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CONTENTS

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Biochemistry of Cancer

By

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Research on cancer is one of the foremost scientific activities of this generation, and constitutes a recognition of this disease not only as an important problem in public health but also as a topic of fundamental scientific interest. This volume is an integration of the results of modern experimental approaches along biological and biochemical lines to the study of cancer. It is intended to be informative, not only to the professional cancer investigator, but to biologists and biochemists in all fields of endeavor, as well as to physicians and medical students.

CONTENTS (Abbreviated):

"This volume represents a vast amount of scholarship and will be indispensable reading for all workers in cancer research, whether savant or tyro. It is destined, no doubt, to become dog eared in scores of laboratories."

Charles Huggins in *SCIENCE*

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Protein-Chemical Aspects of Cancer*

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1. INTRODUCTION

The idea that the abnormal behavior of cancer cells is an expression of abnormalities in their chemical make-up is an old and obvious one, and the early part of this century saw a number of enthusiastic analytical attacks upon this problem by chemical investigators. That these attacks were focussed largely upon proteins is not surprising in view of the predominating position both quantitatively and functionally of this chemical class in the composition of tissue solids. However, when some premature conclusions based on faulty evidence concerning characteristic abnormalities in the amino acid content of tumor proteins were anticlimaxed by further and more firmly founded data indicating absence of an obvious tumor-distinctive amino acid spectrum, interest in this line of attack waned and at the same time other aspects of cancer chemistry, such as those of carcinogenic agents and enzymatic activity, grew in importance as foci of investigative attention. While at present the available evidence speaks against a striking gross difference between "cancer protein" and "normal protein," the conclusion that the proteins of normal and malignant cells are the same is certainly not warranted. At the microscopic level of the modern cytologist, cancer cells definitely reveal morphological abnormalities (18, 32, 110) even though under the low-power lens of the clinical pathologist the individual malignant cell shows no

* Aided by a grant from Mrs. L. Elizabeth Nax.

distinctive properties.[1] Similarly, perhaps, the chemist will not truly enter into the chemical sphere of cancerous protoplasm until he descends to subtler levels of topographical and analytical differentiation than he has been wont to so far. The view that this sphere is one primarily of altered protein chemistry is re-emerging in recent years from many and varied investigative directions, such as immunology, genetics and cytology, virus research and enzymology.[2]

The existing literature pertaining to the protein problem in cancer is widely scattered in time and space.[3] This review represents an attempt to compile the analytical material concerned with the question whether malignant growth is associated with specific changes in protein composition. It has been the writer's intention to bring together related investigations and, with regard to methods and results, to reproduce enough detail to make possible some evaluation of validity and significance.[4] While attention has been directed primarily toward the protein components of the malignant tissue itself, relevant data concerned with the protein chemistry of non-malignant organs of cancerous organisms have not been neglected. In order to limit the scope of the work, emphasis has been on structural rather than functional aspects; that is, composition rather than metabolism has been studied, enzymology thus being excluded, except for aspects bearing directly on the constitutional view point. In chemical terms, the area covered includes proteins and nucleic acids as well as their respective component parts and building stones.

2. PROTEIN CHEMISTRY OF CANCER TISSUE

2.1. Amount and Kind of Protein Fractions

How does cancerous tissue differ from the norm in the distribution of protein fractions characterized by physical or chemical methods of fractionation and analysis? In an early investigation Wolff (205) ground human tissue with sand and pressed the mass at 300 atmospheres, thus obtaining an average of 30 cc. of press juice from 500 gm. of tissue. He determined total nitrogen, total protein nitrogen (on the protein obtained by heat coagulation in the presence of acetic acid), "euglobulin" and "pseudoglobulin" by ammonium sulfate precipitation, and "albumin" by coagulation in the filtrate. Data are given for 7 normal organs (3 livers, 2 ovaries, 2 stomachs) and 18 cancer cases (9 hepatic, 6 mammary, 2 ovarian

[1] Clinically the identification of malignancy rests primarily on tissue architecture, and it has been said that "the microscopic diagnosis of malignancy in the majority of cases is made with a low power lens and a high power brain" (159).

[2] Mann and Welker (134) concluded that their immunological observations "indicate that malignant tissue protein differs from the proteins of normal tissue." v. Euler and associates (56) postulated that "the primary effect of the cancer-forming mutation may be a destruction of those genes which form the growth-regulating enzyme systems" while, on the basis of the literature and their own cytochemical investigations, Caspersson and Santesson (32) assume that in cancer there is "a universal shift toward what are probably more simple proteins, considerably richer than the average in diaminoacids." According to Biesele and his group (18) "the widespread occurrence of polytene chromosomes in cancer indicates their association with the fundamental cancerous change and emphasizes the notion that the many diverse "causes" of cancer must be channeled through the same stream." Jordan (90, 91) pointing to the similarities in the concepts of virus and gene proposed "a multiplication of some genes of a cell as the essential cause of malignant growth" and saw in a hypothetical cancer virus a gene released from the integration of the chromosomal system. Lavik and co-workers (121) on the basis of experiments with carcinogenic hydrocarbons postulate that carcinogenesis is "essentially an accumulation of abnormal protein within the cell," and to Potter (157) the primary abnormal protein consists of an autosynthetic "cancer virus" which is similar to, and therefore competitively blocks the action of, a normal enzyme. The possibility that enzyme-functional abnormalities of cancer may be the results of protein alterations was also stressed by Dieckmann (43). This view is in keeping with the apoenzyme-coenzyme theory of enzyme structure and finds support in recent studies concerning d-amino acid oxidase which (200, 201) demonstrate that the decreased d-amino acid oxidase activity in the liver of cancerous rats is the result of changes in the protein component of the enzyme, the amount of the respective coenzyme remaining unchanged.

[3] For translation of Italian and Russian papers the writer is indebted to Dr. Irene Diller and Mr. Jacob Beller.

[4] In the unavoidably subjective task of selection it has been considered proper not to ignore experimental material merely because it has been obtained 30 or 40 years ago, but on the other hand to omit even much more recent work if it is excessively lacking in detail, if distinctly inadequate methods were used or if, as a result of subsequent investigations, its contemporary significance has become obviously transient. As to the chronological distribution of the publications retained in the bibliography, their average annual rate of appearance for the six decades ending 1945 is as follows:

1885-95	1896-1905	1906-15	1916-25	1926-35	1936-45
0.1	0.2	0.6	1.5	4.3	14.4

In the light of statistics, the protein chemistry of cancer thus appears as a rapidly expanding field, especially since a similar analysis of the literature in the much more active field of organic carcinogenic substances, based on the references quoted in the pertinent chapter of Stern and Willheim's handbook (186), reveals a similar, practically geometric rate of progression.

The numerical data recorded in the text and tables represent in many cases simplifications, rearrangements and recombinations of those found in the original publications.

and 1 gastric). In terms of 100 for the sum of the three protein fractions determined, the results may be summarized as follows:

	Normal Tissue	Cancer	
	Range	Average	Number within range of normal
Euglobulin	19-36	<13	1
Pseudoglobulin	15-32	>16	
Albumin	13-56	71	1

The total protein nitrogen of the extracts shows a tendency to be lower than normal in the cancer tissues (0.16 to 0.81 gm. N, average 0.50, per 100 cc.; against 0.51 to 0.91, average 0.69) while the part of the total protein nitrogen not included in the three separately determined fractions tends to be higher (2.4 to 9.4 per cent compared with 0 to 2.4 per cent).

In evaluating these findings it must be considered that not more than about 2 per cent of the total tissue protein seems to be contained in the press juice, and that much of it is likely to be extracellular (serum) protein (131). If this work indicates increased albumin and decreased euglobulin as a characteristic of malignant cell protein it is supported by Kahn (94) who stated that, according to unfinished investigations, albumins, and especially their most soluble components, predominate in the protein of malignant tumors. Schenck (172) extracted Jensen sarcoma and normal rat liver with cold 1 per cent sodium chloride solution and designated the defatted and dried residue as "tissue protein," while acidification of the extract with acetic acid yielded "nucleoproteid" and heat coagulation of the filtrate gave "combined albumin and globulin." His figures (percentage of fresh weight), obtained on pooled samples are:

	Normal liver	Jensen sarcoma
Tissue protein	14.1	3.3
Nucleoproteid	1.7	0.6
Albumin and globulin	0.6	0.3

They agree with the aforementioned findings to the extent that they suggest the presence in the sarcoma tissue of a large fraction of highly soluble protein not included in the three isolated portions. Caspersson, Nystrom and Santesson (31) likewise speak of an increase of "uncoagulated" protein. Robin (163) compared cancerous livers with normal and tuberculous livers by boiling out the dried tissue successively with ether, water, and alcohol. The quantities extracted vary widely within each group, and only the water-extractable fraction shows a suggestion of definite displacement. When the highly involved (*très atteintes*) and relatively healthy (*relativement saines*) parts of the tumors were analyzed separately it was found that, in terms of the completely extracted residue, the aqueous extract of three normals amounted to 52, 56 and 58 per cent, while in 4 tumors the highly involved areas yielded 38, 40, 46, and 56 per cent, but the relatively healthy parts gave 52, 89, 103 and 108 per cent. Absence of experimental or pathological details limits the value of these data. Furthermore, tissue autolysis is admittedly a factor in the results, the analytical work having been begun about 24 hours after death.

An investigation of Edlbacher and associates (49) concerned primarily with the investigation of purine nitrogen shows for Jensen rat sarcomas (5 cases) an average total nitrogen value substantially lower than that of normal livers (13 cases): 2.5 per cent, against 3.3 per cent, based on fresh weight. Unfortunately, neither fat content of the livers nor size or age of the tumors are reported. Nakahara and collaborators (145) found total nitrogen values of 12.4 to 15.0 per cent (12 cases, average 13.9) in hepatomas, compared with 8.8 to 14.7 per cent (15 cases, average 11.9) in normal rat livers, calculated on an ash (7.7 per cent versus 5.7 per cent average)-free and dry (water 82 per cent versus 71 per cent average) basis. Chisolm (33) saw no significant difference in the total nitrogen values (on a dry basis) of a few specimens of human cancer and its parent tissue.

In connection with protein fractions the work of Klar (100) must be mentioned. It is primarily concerned with an abortifacient protein found in the urine of cancer patients (see section 4.); however, this protein was also found in the cancer tissue itself, and, in smaller concentrations, in some normal organs. It is said to belong to the albumin fraction of tissues.

Kubowitz and Ott (115) subjected rat sarcoma tissue as well as normal muscle to an elaborate sequence of extractions and precipitations designed for the isolation of a specific enzyme protein (pyruvic acid hydrogenase). From 3,000 gm. of "nearly histologically pure" tumor tissue 50 mgm. of pure enzyme protein were obtained, while 1,000 grams of muscle gave 200 mgm. The product obtained represented about 10 per cent of the total initial enzyme activity in the case of the tumor tissue and about 20 per cent in the case of the muscle tissue. Elementary analysis, absorption spectra, specific rotation, pH-activity curves and inactivation rates revealed no difference between the two preparations.

2.2. Physicochemical Data on Protein Composition

Vlès and de Coulon (193) determined the pH of fresh tissue by crushing small samples with indicators between slide and cover glass. The tissue was

then frozen in liquid of its own pH, ground, and small portions of the powder were added to solutions of different pH. Approximate isoelectric points were obtained by subjecting the resulting suspensions to electrophoresis and determining rate and direction of migration by visual observation as well as by precipitation with alcohol-acetone. The tissue pH values of mouse muscle were approximately 6 while in cancers (transplanted sarcomas and spontaneous mammary carcinomas) slightly more acid values (pH 5 to 6) were found. The predominating isoelectric point in normal mouse muscle was approximately 0.8 units higher than the pH, while in cancer tissue (peripheral, non-necrotic parts) the difference was 1 and larger. Even greater differences between the two acidity values were found in the normal muscle tissue of carriers of malignant tumors. On the basis of their comprehensive study (including the observation of higher isoelectric points in cases of contagious diseases, and in pregnancy of an initial shift toward higher values followed by a gradual return to the normal level before delivery) the authors suggest that a rise in the isoelectric point of tissue may be the result of antibody formation while a decrease may indicate the presence of autolysis products. They conclude from quantitative considerations that cellular proteins rather than interstitial serum proteins are primarily responsible for the phenomena observed. The specific and variable errors caused by proteins in colorimetric pH determinations ("protein error") seriously limit the significance of this work.

Results obtained by Solowjewa (182) point in a similar direction, although the numerical values are different. The method used here was that of Pischinger (155) in which alcohol-fixed tissue slices are stained with methylene blue in buffers of increasing pH, and the pH value at which staining of any particular cytological structure just begins is considered as an approximation of its isoelectric point. According to Solowjewa's data the nuclei of epithelial and connective tissue cells in cancerous tissue (both human and mouse) show a slightly more acid isoelectric point (pH 3.2 to 4.0, average 3.4) than the nuclei in the corresponding (mammary gland, uterus, stomach) normal tissues (pH 3.6 to 4.0 average 3.9). For cytoplasm, values of pH 5.0 to 5.5 (average 5.3, cancerous), and pH 5.2 to 5.5 (average 5.4, normals) are shown.

It is interesting to compare these *in vitro* determinations with the electrometric pH measurements performed *in vivo* by Kahler and Robertson (93). In living animals they found in normal livers an average value of pH 7.39 (12 cases, ranging from pH 7.18 to 7.51), compared with an average of pH 6.99 (10 cases, pH 6.81 to 7.10) in subcutaneous transplants of hepatoma 31 (originally induced by butter yellow feeding).

The result that these varied investigations have in common is a higher acidity in cancer tissue than in normal tissue. An increased nucleic acid content of the malignant tissue (see section 2.5.2.) is perhaps the most likely explanation of these phenomena, and it seems unfortunate that no parallel determinations of tissue pH and nucleic acid content are available.

2.3. Nitrogenous Intermediates

2.3.1. *Peptides, amino acids, creatine, etc.*—In a comparison of carcinomatous, sarcomatous and noncancerous human lungs, Lustig (132) found that in the protein-free filtrate the "albumose and peptone" nitrogen fraction (precipitated by tannic acid and acetic acid) was consistently higher in four malignancies (2.1 to 9.4 per cent of the total nitrogen) than in four normals (1.4 to 1.9 per cent), as was the diamino nitrogen fraction (precipitated by phosphotungstic acid) (0.6 to 1.4 per cent compared with 0.2 to 0.3 per cent). Nakahara and associates (144) found that in transplantable rat hepatomas, although total nitrogen was only slightly elevated (143), non-protein nitrogen, amino nitrogen, creatine, urea and uric acid were approximately twice as high as in normal livers when calculated on a dry basis. When calculated in terms of fresh tissue weight the differences were insignificant and in this respect the Japanese results are confirmed by a recent investigation of Greenstein (73) who using the specific enzymatic method of Miller and Dubos (137) found no differences in the creatine content of fresh normal, regenerating and cancerous tissues of either the mouse or the rat. However, the absolute values of the mouse were only one-half as large as those of the rat. While Nakahara's figures for total creatine are similar in magnitude to Greenstein's, the essential feature of the Japanese findings —high extractives nitrogen in terms of total nitrogen —cannot be verified because of absence of total nitrogen figures in Greenstein's study.

In a comparison of 10 tumors, which were Jensen rat sarcomas and "in a few cases" Ehrlich mouse carcinomas, and 13 rat livers, Annau and Gözsy (4) found in the former 0.011 to 0.032 (average 0.021) per cent of free arginine and in the latter 0.000 to 0.005 (average 0.004) per cent. Increased free arginine not only in tumor tissue (0.48 per cent, on the basis of the dry, ether-extracted residue) but also in the normal muscle of tumor-bearing rats (0.52 per cent, compared with 0.15 to 0.17 per

cent), has been reported by Klein and Ziese (104) who emphasize that, if it were not for the high arginase activity of tumor tissue, the difference would presumably be even larger.

2.3.2. *Glutathione.*—Interpretation of data concerning the tripeptide glutathione is rendered difficult through the multiplicity of analytical methods used and in most cases their actual non-specificity. An investigation in which an apparently reliable method (see section 3.1., Table XVI, for a comparison of normal liver values obtained by different methods) was applied to transplantable (Ikabo's) rat hepatomas and, for comparison, livers of normal albino rats, is that of Fujiwara, Nakahara and Kishi (62). These authors found, on the average, approximately 40 per cent more glutathione in the hepatomas than in the normal livers:

	Number of determinations	Glutathione, % of fresh tissue	
		Average	Range
Hepatoma	16	0.243	0.175-0.307
Normal Liver	16	0.171	0.125-0.234

On the other hand, Kinosita (99) found in primary butter yellow hepatoma 11 per cent less glutathione than in the livers of normal rats (his value for the latter was 0.270 per cent) while Iki (88), also with butter yellow hepatomas, found no difference between hepatoma and controls (0.214 and 0.217 per cent respectively). Details of these investigations were not available to the reviewer. Greenstein (69), tentatively identifying his "cysteine" values, determined in aqueous tissue extracts, with glutathione, obtained 0.159 and 0.121 per cent respectively for normal rat liver and hepatoma 31 transplants. Other data on glutathione are discussed in section 3.1. It may be noted here that the results of independent investigations (194, 208) agree in finding a relatively, though not abnormally, high glutathione content in living carcinoma and sarcoma tissue, while in necrotic cancer tissue the value is almost negligibly small.

2.4. Ordinary Protein Components

2.4.1. *Basic amino acids.*—The search for characteristic features in the amino acid distribution of cancer proteins was begun soon after the early methods of amino acid fractionation were developed. Neuberg (145) found no unusual values for the diamino and monoamino fractions (determined by the Hausmann phosphotungstic acid method) of human liver metastases from a primary stomach carcinoma. The values of 35.8 and 64.2 per cent of the total (fat-free) nitrogen were compared with data of 35.8 and 62.5 for glue, 33.4 and 60.3 for serum albumin, and 27.3 and 63.1 for casein.

Wells and Long (198) gave similar Hausmann results. For basic and monoamino nitrogen they obtained 24.8 and 65.5 per cent in a liver nodule secondary to an ovarian carcinoma, compared with 31.4 and 60.2 per cent for normal liver. More recently Bolaffi (23) extracted tissue proteins with 10 per cent sodium chloride and 0.1 per cent sodium hydroxide and obtained in the resulting extracts the following percentages of the total nitrogen precipitable with phosphotungstic acid, after acid hydrolysis:

	NaCl extract	NaOH extract
Spleen (horse)	29	30
Liver (calf)	50	10
Kidney (calf)	..	22
Adenocarcinoma (mouse)	34	25

Considerable attention was attracted by an investigation of Kocher (106) in which the diamino nitrogen percentages of 5 different human cancers were determined by a modified Kossel-Kutscher (silver ion, phosphotungstic acid) method. Unfortunately, Kocher compared his results (26 to 31 per cent) with those obtained by Wakeman (196), who "employed essentially the methods I have used" and reported values between 8 and 16 per cent for a variety of normal tissues of man, dog, horse and ox. The resulting conclusion concerning a doubling of the diamino acid content in malignancy, born of an unwarranted faith in methods, is still being cited in some recent publications, even though it was promptly challenged by an elaborate study of Drummond (46). By the Van Slyke method Drummond investigated the nitrogen partition in tissue protein hydrolysates obtained from 41 cancer cases (20 human breast, 1 human lymphosarcoma, 20 Rous chicken sarcoma), 12 normal human and 12 normal chicken tissues. The material was obtained by coagulation of minced tissue with slightly acid boiling water, precipitation of soluble protein by alcohol, followed by ether extraction. Drummond's very voluminous data show that the diamino fraction may differ widely between different tissue types: e.g. 27 per cent for normal mammary gland tissue, and 38 per cent for normal spleen. Lymphosarcoma gave the same value as spleen, while in primary breast cancers values up to 30 per cent were found. The chicken tumors averaged 34 per cent, compared with 28 per cent for normal chicken tissues. However, in the latter case, the increase cannot be assigned to the malignant modification, as the author himself pointed out, because normal connective tissue as the true histological analogue of the Rous sarcoma (see reference 14) was not studied. As to the individual diamino acids, arginine seems to be the least variable of the three, while there is a suggestion in the data

that increased malignancy, both in the human breast carcinomas and the chicken sarcomas, is associated with an increase in the relative amount of histidine. Drummond's findings are illustrated by the selected data given in Table I. Although the trends in the histidine and lysine averages of the breast groups are consistent, in evaluating their possible significance the broad range covered by the individual values within the groups must be considered: the average range within the groups (excepting the normals) is 5 percentage points, e.g. for arginine in general fibrosis, the minimum value is 8.7, the maximum 14.0, and the average 10.6. Furthermore, the non-amino nitrogen fraction of the neutral amino acids, indicative of the prolines, tends to be lowered in the malignant tissues. Drummond's conservative interpretation of the observed trends is that both increased diamino fraction and decreased nonamino fraction are primarily conditioned by the ratio between cellular (nucleated) and stromatous (connective tissue) material, the latter being characterized by high concentrations of the prolines and the former by being rich in basic amino acids.

Table I: Basic Amino Acid Content of Tissue Proteins

From Drummond (46)

	Number of determinations	Average per cent of total N			
		Arginine N	Histidine N	Lysine N	Basic amino acid N
Rous sarcomas*					
Slow growing		12.1	9.3	11.7	33.8
Medium growing		10.8	10.1	13.0	34.9
Rapidly growing		11.5	10.6	12.0	35.8
Human breast					
Normal	2	10.1	3.7	12.9	26.7
General fibrosis	4	10.6	6.7	9.6	27.7
Slow growing scirrhus carcinoma	6	11.2	5.9	11.2	28.3
Rapidly growing tumors	3	10.1	7.3	12.9	30.3
Secondary deposits in glands	6	10.5	8.1	13.3	31.9
Human spleen	1	12.5	13.5	11.0	38.1
Human lymphosarcoma	1	11.9	8.9	16.3	38.2

* 20 cases.

Schenck's data (172) on the "tissue protein fraction" (insoluble in 1 per cent sodium chloride) and a "nucleoproteid" fraction (precipitated from the saline extract by acidification with acetic acid; see section 2.1.) are summarized in Table II. The "tissue protein fraction" of the malignant species may have an increased lysine content in accordance with some of Drummond's data, but with regard to histidine there is contradiction. However, according to the yield figures Schenck's two sarcoma fractions probably represent no more than one-third of the total tissue protein (see section 2.1.). A later study by Rosedale (167) indicates a low lysine content for carcinoma, but the absolute values are much lower than those of Drummond and Schenck. Rosedale analyzed minced, coagulated tissue, after removal of extractives by boiling 0.003 *M* acetic acid, by the Kossel and the Van Slyke methods, with essentially similar results. He reported the low value of 2.5 per cent (according to Van Slyke; 3.0 per cent by the modified Kossel method) lysine-nitrogen for breast carcinoma, compared with an average of 7.2 ± 0.7 per cent (6.5 to 8.4) for some other tissues (human arm and foot; muscle, uterus and fetus of pig), while pig placenta gave the intermediate value of 3.9 per cent.

Table II: Basic Amino Acid Content of Tissue Protein Fractions

From Schenck (172)

	"Tissue protein"		"Nucleoproteid"	
	Rat liver	Jensen Sarcoma	Rat liver	Jensen sarcoma
Yield, per cent of fresh tissue	14.1	3.3	1.7	0.6
Per cent of total N				
Total diamino N	36	35	35	34
Arginine N	8.2	10.2	6.6	10.0
Histidine N	20.0	16.0	28.9	24.5
Lysine N	8.1	9.0		

Arginine which shows no definite trend in Drummond's data is increased in malignancy according to Schenck, and Rosedale's figures point in the same direction: breast carcinoma gave 9.1 per cent arginine-nitrogen, while the control tissue mentioned averaged 8.6 ± 0.3 per cent (8.1 to 9.0). A high arginine-nitrogen value of 11.3 per cent for pig placenta pointed to a similarity of that tissue with malignant species, as did its low lysine value. The

fact that, contrary to placenta, pig fetus fell within the normal range, both in terms of arginine and lysine, needs verification and elaboration because of its relation to other embryonal aspects of cancer and its possible bearing on the cancer theories based thereon.

Zbarskii and associates (211) obtained tissue protein by boiling and washing the dissected tissue with water and combining the residue with the precipitate obtained from the aqueous extracts by boiling with acetic acid. Diamino acids were determined by Cavett's modification of the Van Slyke method, the arginine content being verified by the Sakaguchi method "which gave concurring results." The results were corrected for moisture, ash and fat content. Comparison in this investigation is between pooled specimens of Ehrlich carcinoma and Vienna sarcoma of the mouse, and liver and muscle protein of the same animal. Table III shows a digest of the numerical results. Some of the malignant specimens are from animals injected with basic amino acids, and since the resulting analyses show no discernible effect of these treatments the data are included in the averages. The only definite finding appears to be that in the carcinomas, and less unequivocally in the sarcoma protein, the arginine value is higher than in that of muscle or liver.

the total arginine value of 13 livers was 3.4 ± 0.5 per cent (average deviation; range 2.2 to 4.5) while that of 10 tumors—Jensen sarcomas, and, "in a few cases" (!) Ehrlich mouse carcinomas—was 4.5 ± 0.4 per cent (3.4 to 5.4). The corresponding values for soluble arginine were 0.004 per cent (0 to 0.005) for the livers, and 0.021 per cent (0.011 to 0.032) for the tumors. Contrary to Klein and Ziese (104) Annau and Gözsy found no arginase in the tumors, but substantial amounts in the livers, and saw therein the explanation for the different levels of soluble arginine. The total arginine values of the two investigations are in good agreement, but the soluble arginine figures in the former study are 20 times as high as in the latter. Further exploration of the nature of this difference may be useful since the higher results were obtained by hot water extraction and the lower ones by cold trichloroacetic acid treatment.

In summarizing the evidence concerning the individual basic amino acids it appears that five investigations (172, 167, 211, 104, 4) agree that arginine is increased in cancer protein, while regarding histidine and lysine conflicting conclusions have been reached. Methodological errors are undoubtedly large in all of these studies.

New evidence concerning embryonal aspects of

TABLE III: BASIC AMINO ACID CONTENT OF TISSUE PROTEINS

From Zbarskii (211)

Tissue	Number of pooled specimens†	Per cent of total protein nitrogen* (with average deviation) Diamino N	Arginine N	Histidine N	Lysine N
Carcinoma	9	33.7±0.9	15.9±0.3	4.5±0.2	13.2±0.3
Sarcoma	3	31.6±0.7	14.3±0.7	4.4±0.2	12.8±0.2
Muscle	6	30.4±0.9	13.9±0.5	4.2±0.2	12.4±0.5
Liver	6	30.1±1.2	13.9±0.3	4.9±0.5	11.2±0.7

* The protein was hydrolyzed "for 3 hours under a pressure of 3 atmospheres" with 20 volumes of 20 per cent HCl.
† Two complete determinations were run on the hydrolysate of each specimen.

There are several more recent investigations dealing with arginine specifically and utilizing the colorimetric Sakaguchi method. Klein and Ziese (104) determined both free (water soluble) and total arginine in freshly dehydrated, ether extracted tissue. Because they did not compare their malignant tissues with homologous normal tissues but with muscle of normal and tumor-bearing animals, their results are primarily of interest in connection with the remote effects of malignant growth (see section 3.1.3.).

Annau and Gözsy (4) compared Jensen rat sarcoma with liver in regard to arginine content. They applied the Sakaguchi method to the trichloroacetic acid-insoluble part of the tissue, and to the trichloroacetic acid wash liquid. In terms of dry tissue, malignant tissue has recently appeared from different directions (78, 156, 68); and it seems worth recalling that in a chemical study of the embryonic growth of the pig, Wilkerson and Gortner (202) found that embryonal development is accompanied by decreasing arginine and histidine content while lysine increases. In stressing the relationship between embryonic and tumor chemistry these authors also stated that "the more embryonic a tumor tissue the more arginine is found in its contents."

2.4.2. *Protein sulfur.*—A simple index of the amino acid composition of protein preparations is the sulfur content since apart from possible minor amounts of sulfate it stems from the sulfur-containing amino acids. Unfortunately some of the methods

used for its determination have in recent years been shown to give low values, owing primarily to the resistance of methionine sulfur to complete oxidation.

Nakahara and associates (143) compared a transplanted rat hepatoma with normal liver tissue. Use of entirely parenchymatous material and actively proliferating tumor cells was stressed and a good sulfur method[5] was used. The whole, minced and dried tissue was analyzed. The results, calculated on an ash-free basis, appear to indicate a significantly increased sulfur percentage in the hepatomas. For 16 specimens the average is 1.24 ± 0.17 (average deviation), compared with 0.98 ± 0.12 for 14 normals. However, when the results are expressed in terms of the atom per cent ratio of sulfur and nitrogen, the difference vanishes: 3.7 ± 0.3 compared with 3.7 ± 0.5, and it seems likely that the absolute difference reflects differences of fat or carbohydrate content rather than of protein composition.

Brown and Klauder (27) gave results purporting to show that the sulfur content of dried mouse carcinoma varies in proportion to growth rate. For 3 slowly growing tumors they reported values of 0.40 to 0.47 per cent S, for 3 normally growing ones, 0.52 to 0.61 per cent, and for 3 of very rapid growth, 0.69 to 0.74 per cent. These results cannot be further evaluated because no other data are given. The method of sulfur determination used (187) would seem to be a satisfactory one. In a subsequent paper dealing with human material (102) the same authors state that "a study of the percentage of sulfur in malignant tissue from various viscera did not show uniformly a greater percentage of sulfur than that contained in the organ from which the malignant growth arose. Results of the study were not as striking as those obtained in the . . . similar study (27) of mouse carcinoma." Unfortunately in their second study the authors used a modification of the Benedict-Denis sulfur method which has been shown to yield less than 40 per cent of the methionine sulfur present (151).

2.4.3. *Sulfur amino acids.*—The most important investigations concerning the sulfur content of cancer protein appear to be those of Greenstein and his associates. By alkaline extraction of fresh liver tissue in the presence of 0.5 *M* potassium chloride (in order to minimize solution of other liver proteins), precipitation at pH 4, repeated reprecipitations under similar conditions, and final extraction of lipids, they obtained a nucleoprotein fraction (N =15.5 to 16.0 per cent, P = 0.7 to 0.9 per cent) of nearly constant P : N atom per cent ratio (2.0 to 2.5) and representing about 5 per cent of the total tissue protein. In addition to normal rat liver (70) analogous preparations were made from Jensen sarcoma (71) and transplanted hepatoma 31 (72). Table IV is constructed from the authors' data. There is a possible suggestion in these figures of a non-cystine, non-methionine sulfur fraction in the normal preprarations which tends to be replaced by cystine sulfur in the cancerous product. With regard to sulfhydryl groups made reactive in these products by denaturation with guanidine, only Jensen sarcoma differed from the non-malignant tissue; approximately 20 per cent of its total cystine sulfur showing this property, compared with none in the other two products. Later (69, 76, 77) aqueous extracts containing a more substantial fraction (7 to 9 gm. per 100 gm. of fresh tissue, i.e. of the order of 50 per cent of the total) of the total tissue protein, were prepared from adult rat liver, hepatoma 31, and in addition regenerating and fetal liver. The S : N atom per cent ratio was substantially higher in these extracts than in the nucleoprotein fractions, and practically identical in all four cases: 5.7 or 5.9, corresponding to an estimated protein-sulfur content of 2.1 per cent; approximately one-tenth of it may be contributed by glutathione (77). However, the identical sulfur content conceals what appear to be important differences in the sulfur distribution. Whereas in the normal and regenerating liver, cystine sulfur accounted for 80 and 79 per cent respectively of the total sulfur, the corresponding figures were only 53 and 49 per cent in the preparations of fetal and malignant origin. The remaining sulfur was in all cases substantially accounted for by methionine. These findings recall the results of Graff and Barth (67) on sulfur partition in the developing frog embryo; while total sulfur remained constant cystine sulfur increased from an initial low value, suggesting that the early embryonal protein has a high methionine content. A similar relation—methionine as a de-

TABLE IV: SULFUR DISTRIBUTION IN TISSUE NUCLEOPROTEINS

From Greenstein (70-72)

Nucleoprotein from	Atom per cent Ratio S : N	Total sulfur % Accounted for by Cystine	Methionine
Rat liver	3.3	30	55
	3.4	31	55
Jensen rat sarcoma	3.7	38	55
	3.2	41	58
Rat hepatoma 31	3.0	36	59

[5] Fusion with potassium hydroxide and potassium nitrate according to Liebig; this method gave for casein, which contains about 10 times as much methionine as cystine, values (82) identical with modern results (95).

velopmental precursor of cystine—may be deduced from data of Block and Lewis (22) on the cystine and total sulfur content of cattle hair; in the hair of the youngest animals the cystine content corresponded to only 80 per cent of the total sulfur, and this figure gradually increased toward close to 100 per cent with increasing age of the animals. In the light of these few scattered observations, the methionine-cystine ratio and the conversion of the former into the latter appear of considerable developmental interest. Also, Greenstein's observations lend support, from a chemical angle, to the theories that regard cancer in terms of embryonal residues or regression to a cellular embryonic state.

One of Greenstein's investigations (69), devoted particularly to the liberation of sulfhydryl groups on denaturation, shows that in this respect the aqueous extracts reveal no consistent difference between malignant and normal tissue; while in hepatoma 31 the latent sulfhydryl fraction was substantially smaller, both in absolute amount and relatively (in terms of total cystine sulfur), than in normal rat liver, the study of two types of mouse hepatomas revealed no difference in comparison with the corresponding normal livers.

2.4.4. *Tryptophane.*—Among other specific amino acids the role of tryptophane in cancer tissue has been variously investigated Fürth, Kaunitz and Scherf (60) studied the protein of human non-cancerous and cancerous livers. The tissue was dried, extracted with ether, pulverized, and extracted with boiling water, or precipitated with trichloroacetic acid. If the average tryptophane content (2.6 per cent) of normal human liver protein is taken as 100, the actual values ranged from 85 to 123, and similar values were obtained from pathological livers, such as cases of cholangitis and fatty degeneration. While carcinomatous livers also fell into this range, melanosarcoma metastases in the liver gave values of only 23 to 58. The analytical method used in this work (Fürth-Lieben) was also employed by Edlbacher and Baumann (51) and found by them to possess an accuracy of ± 4 per cent. The latter reported total nitrogen and tryptophane determinations on rats bearing 3 weeks old Jensen sarcomas, and normal animals. Their figures show that the tryptophane fraction of the total nitrogen is 32 per cent lower in the tumors than in the unaffected parts of the livers, but nearly normal in the necrotic areas. The fact that no individual values but only the averages from 6 tumor and 6 normal animals are reported detracts from the usefulness of this study. Furthermore, the authors' conclusion that the tryptophane content of tumor and necrosis is identical, is misleading because it is based on tryptophane values expressed in terms of percentage of fresh tissue, a mode of calculation repudiated by the same authors in another study (50). Two similar but completely separate investigations were reported by Lang (116) and Rosenbohm (168) respectively. While Lang's values for the tryptophane nitrogen fractions are substantially lower than those of Edlbacher and Baumann (51) it is remarkable that his average from 32 Jensen sarcomas is 30 ± 3 per cent (cf 32 per cent [51]) lower than the average from 20 normal rat livers while the necrotic areas gave values within the normal range in both studies. Lang also records a comparison of 4 human carcinomas with 2 specimens of human muscle, with the former average being lower by 40 per cent. Rosenbohm's values (168) though obtained by a different method are similar in magnitude to those of Lang (116), and his average tryptophane value of 14 Jensen sarcoma proteins is 24 ± 4 per cent lower than that of 5 nonsarcomatous liver proteins. The necrotic areas again gave values within the normal range. Tryptophane values of 3 human mammary carcinomas (without controls) are even lower than those reported by Lang (116). According to the previously mentioned (see section 2.4.1.) investigation of Zbarskii (211) the tryptophane content of Ehrlich mouse carcinoma protein is slightly, although hardly significantly, lower than that of liver or muscle protein. The 23 complete sets of data on individual tissue specimens do, however, reveal an apparently significant positive correlation between tryptophane and histidine values—the probability of randomness (P) being less than 0.01 (181)—and a similarly significant negative correlation between tryptophane and arginine values ($P<0.02$).

Because of the confusion in methods, no numerical results are listed here; however, further investigation of tryptophane content is clearly indicated, both with regard to different types of malignant growth and with regard to different cytological and chemical fractions. In view of Mirsky and Pollister's (138) statement that nuclear histones are tryptophane-free the low tryptophane values reported in the three studies mentioned could be attributed to a high nuclear content of the malignant tissue; and if the chemical investigation had been accompanied by a histological study such a surmise could have been verified.

The investigations of Greenstein and his associate on Jensen rat sarcoma and hepatoma 31 (70-72) have already been discussed in connection with sulfur compounds. Tryptophane values of their nucleoprotein fraction are higher than those given by Lang

(116) and Rosenbohm (168) for the whole tissue protein, and the products of malignant origin give values (also in the case of tyrosine) identical with those of normal origin. However, it must be remembered that Greenstein's nucleoprotein is a quite different entity from that of Mirsky and Pollister (138), obtained by another procedure and of much lower phosphorus content (P : N atom per cent ratio 2.3 versus 11).

2.4.5. *Other amino acids.*—As to amino acids other than those mentioned so far, very little work is available. In 1910 Abderhalden and Medigreceanu (1) "determined" by isolation methods tyrosine, glutamic acid and glycine in three cases of cow's liver carcinoma, one of rat sarcoma and one of mouse carcinoma. In terms of nitrogen fraction (of the whole tissue dried to constant weight at 120° C.) the values found for tyrosine were 1.1 to 1.2 per cent, for glutamic acid 7.2 to 9.0 per cent, and for glycine 1.9 to 3.0 per cent. Although no normal tissues were included in this investigation the values obtained were considered to be within the normal range by reference to other work of the same authors. Far superior analytical methods have since been developed but no related work is available, with one exception. A publication of Kögl and Erxleben appearing in 1939 (107) and dealing with the optical rotation of amino acids isolated from tissue, purported to show that malignant tissue differed from non-malignant in that it contained appreciable quantities of the optical *d*- ("unnatural") series of amino acids, with glutamic acid exhibiting the most drastic abnormality in this respect. Many investigators followed this lead, with the result that today more than 100 papers bearing on the issue raised by Kögl and Erxleben have appeared. Nevertheless the correctness of their assertion remains a matter of controversy. Although definitely within the province of this review a detailed discussion of the problem may well be dispensed with because the subject is thoroughly covered in several readily accessible recent digests (123, 175, 29). Only one aspect, of direct bearing on quantitative amino acid distribution, should be recorded here; namely data of Kögl and associates (109) obtained by the isotope dilution method, which theoretically gives absolute results. According to this method hydrolysates of malignant tissues not only contain a substantial fraction (average 18 per cent) of their glutamic acid in the *d*-form but also contain approximately 16 per cent more total glutamic acid than corresponding normal tissues: 8.7 to 11.0 per cent of the total protein nitrogen versus 7.4 to 9.3 per cent.

Tyrosine figures for one specific protein fraction are contained in the work of Greenstein and collaborators (71, 72) on "nucleoproteins" (see section 2.4.3.) obtained from liver and Jensen sarcoma. Substantial identity between both types of tissue was found in tyrosine content (nitrogen fraction 1.8 to 1.9 per cent) as it had been in regard to the other components studied.

2.4.6. *Protein phosphorus.*—As an indication of the need for more information rather than for their factual significance, mention is made of data by Euler and Schmidt (55) on phosphoprotein content. By an established method of differential analysis (mild alkaline hydrolysis of the thoroughly extracted tissue) they found in liver and embryos of the rat, calf thymus and fish testicles, phosphoprotein phosphorus values ranging between 0.012 and 0.017 per cent (in terms of fresh weight) while a metastatic liver carcinoma contained only 0.006 per cent. The possible significance of this low value, compared with the figure of 0.016 per cent for embryonic tissue, is seen by the authors in the fact that in regard to many other analytical and functional chemical characteristics malignant and embryonic tissues appear to be similar. However, in comparing normal rat liver and rat hepatoma, Schneider (174) recently did not find significantly different phosphoprotein phosphorus values (0.024 and 0.018 per cent respectively).

2.4.7. *General.*—It seems well to bear in mind, in evaluating the findings enumerated in this section, that identity of one protein fraction (or several) obtained from two kinds of tissue does not prove identity of the protein spectrum of the two tissues, nor does quantitative equality of one amino acid fraction (or several) obtained from two protein fractions prove identity of the amino acid spectrum of the two proteins. Thus while presumably plasma albumin of man represents a single protein entity (38) human plasma as a "tissue" may vary over a wide range in its protein spectrum and these variations may be of great physiological importance, e.g., the immunological properties of the blood globulin fractions (38). The need for extreme reserve in extrapolating from identity of amino acid content to identity of proteins is also well illustrated by plasma protein data; e.g. while human and bovine albumin were found to be substantially identical in regard to 6 amino acids, nevertheless substantial differences existed in 7 others (25). One may also recall the earlier results of Sörensen, cited by Rimington (161), who showed that serum protein fractions may differ greatly in solubility even though on the basis of their content of several amino acids they may appear identical; the data of Knight and Stanley (105) on different virus strains, which though identi-

cal in tyrosine content, differed greatly in tryptophane and phenylalanine; and the investigations of Block (21) and Beach and associates (8) indicating that the hemoglobins of different mammalian species are substantially identical with regard to the 3 basic amino acids but differ markedly in methionine and cystine content.

2.5. Nucleic Acids, Their Components and Combinations

2.5.1. *Nucleic acid phosphorus.*—The available evidence concerning kind and amounts of nucleic acid compounds in cancer tissue is no more conclusive or complete than that on amino acid distribution. Determinations of total phosphorus have repeatedly been used as a clue to the nucleic acid content of tissue. The validity of such determinations is impaired to variable degrees by the presence of phospholipids, phosphorylated amino acids and other phosphate esters. Because phosphoprotein phosphorus presumably is a minor quantity in tissue proteins (40) complete extraction of lipids probably removes the most important sources of non-nucleic acid phosphorus inasmuch as inorganic and acid soluble phosphate represent primarily metabolic derivatives of nucleic acid phosphate. Furthermore, the significance, in terms of the composition of the native tissue, of many values for free and loosely bound forms of phosphate is uncertain because of the rapid autolytic processes involving nucleotides (24, 13, 98, 40, 39).

In the previously discussed work of Nakahara and collaborators (143) on liver and hepatoma there appears an absolute increase in phosphorus in the hepatomas. However, just as in the case of the sulfur values, the difference tends to disappear if the results are viewed in terms of the atom per cent ratio of phosphorus to nitrogen: 4.9 ± 0.2 (for the hepatomas) compared with 4.5 ± 0.2. In a later study (61) on new but biologically identical material the same authors determined, in addition to total phosphorus, the lipid, acid soluble and inorganic subfractions, and found that neither average lipid phosphorus nor protein phosphorus showed significant differences. Lustig (132) investigated 3 carcinomas and 1 sarcoma of the human lung, together with 3 specimens of normal lung tissue. By heat coagulation of the whole tissue with 10 per cent sodium sulfate solution acidified with acetic acid, and differential determination of non-protein components he obtained values for the total tissue proteins. In addition, a "nucleoprotein" fraction was prepared by acid heat coagulation of a tissue extract obtained by extraction with 0.1 *M* sodium carbonate in the cold. The following values for the atom per cent ratio of protein phosphorus to protein nitrogen were obtained (yields are not reported):

	Carcinoma			Sarcoma	Normal lung			
Total protein	1.59,	1.19,	1.08	1.00	0.75,	0.85,	0.57,	0.85
'Nucleoprotein'	1.54,	1.85,	1.89	1.37	1.28,	2.08,	2.33,	1.56

Table V: Total Protein-Bound Phosphorus, as Percentage of Dry Tissue

Malignant tissue				Control tissue				
Type of tissue	No. of specimens	Degree of variation	Av. value	Av. value	Degree of variation	No. of specimens	Type of tissue	Reference
Jensen rat sarcoma	6	0.52-0.84*	0.68	0.34	0.27-0.38*	8	Normal rat liver	13
Butter yellow hepatoma (rat)	8	0.51-0.68*	0.58	0.35	0.29-0.44*	11	" " "	173
Butter yellow hepatoma (rat)	7	0.38-0.65*	0.49	0.31	0.18-0.38*	5	" " "	42
Spontaneous hepatoma (mouse)	4	0.30-0.53*	0.44	0.41	0.28-0.52*	4	Normal mouse liver	42
				0.45	± 0.01†	14	Normal rat liver (stock diet)	41
Butter yellow hepatoma (rat)	5	± 0.04†	0.54	0.51	± 0.01†	6	Normal rat liver (control diet)	41
				0.51	± 0.01†	9	Regenerating rat liver	41
				0.91	± 0.10†	8	Embryonic rat liver	41
Chemically induced fowl tumor	5	± 0.02†	0.53	0.13	± 0.02†	4	Adacent muscle (fowl)	39‡
Rous sarcoma	12	± 0.05†	0.46		0.12-0.41*		Brain, heart, liver, nerve (fowl)	39‡
Human cancers§	5	0.14-0.53*						39‡

* Range of values.
† Standard error.
‡ Data represent percentages of extracted tissue residue.
§ Lymphosarcoma, hypernephroma, secondary carcinoma of colon, fibrosarcoma, carcinomatosis of omentum.

The obvious interpretation would be that the higher relative phosphorus content of the proteins of malignant growths is due to their higher nucleoprotein content. However, whole lung tissue may not be the proper standard of comparison for lung tumors (79).

A number of recent investigations give data on the "protein-bound phosphorus" (170) remaining after extraction of lipids and acid soluble phosphorus components. The accompanying tabulation (Table V) summarizes these data. The figures represent per cent phosphorus, in terms of dry weight, of the original tissue or of the extracted tissue residue. On the basis of the available data Davidson and Waymouth (39) consider it "justifiable to conclude that rapidly growing cellular tumors have, in general, like embryonic tissues, a high nucleic acid content but that it is obvious from the figures obtained from the human tumors that this does not hold in all cases. When a tumor contains large amounts of fibrous tissue, for example, the nucleic acid content, as might be expected,[6] is not high." Caution in drawing final conclusions seems indicated as long as chemical analysis is not accompanied by histological analysis. Rosenthal and Drabkin (171) found that the amount of neoplastic tissue in 5 different rat tumors ranged from 34 to 100 per cent (average 72 per cent), the remainder being connective tissue, while in 9 human cancers neoplastic tissue amounted to 30 to 95 per cent (average 58 per cent).

2.5.2. *Purines, nucleosides, nucleotides.*—As another measure of nucleic acid compounds the determination of total purines has been used. After mild hydrolysis and protein removal a precipitate of cuprous purine compounds is obtained and its nitrogen determined. The available data have been summarized in Table VI.

TABLE VI: PURINE NITROGEN CONTENT OF TISSUE

	Number of specimens	Purine N as % of total N (with average deviation)	Reference
Normal liver, adult rat	1	5.7	54
Normal liver, young rat	1	5.1	54
Normal liver, rat embryo	2	7.0 ± 0.3	54
Jensen rat sarcoma	8	6.9 ± 0.8	54
Jensen rat sarcoma	5	6.3 ± 0.2	49
Normal liver, rat	13	4.6 ± 0.2	49

TABLE VII: AMOUNTS OF PURINE SUBFRACTIONS IN TISSUES
From Barrenscheen and Peham (7)

Type of tissue	No. of specimens	Purine N (% of wet tissue, with average deviation)				Ratio of 3 forms of purine N (total=100) in order of increasing complexity (b):(c):(d)
		Total purines (a)	Non-nucleotide purines (b)	Soluble nucleotides (c)	Nucleoprotein purines (a)-(b)-(c) (d)	
Man: mammary carcinoma	2	0.050	0.025 ± 0.006	0.013 ± 0.001	0.012	50:26:24
Man: stomach carcinoma	1	0.079	0.021	0.013	0.045	27:16:57
Mouse: Ehrlich adenocarcinoma	2	0.125 ± 0.021	0.016 ± 0.001	0.018 ± 0.005	0.091	13:14:73
Rat: Jensen sarcoma	1	0.143	0.012	0.015	0.116	8:11:81
Rat: Flexner sarcoma	1	0.121	0.017	0.008	0.096	14: 7:79
Chicken: Rous sarcoma	1	0.079	0.014	0.016	0.049	18:20:62
Warm blooded animals: striated muscle	5	0.069 ± 0.006	0.011 ± 0.001	0.050 ± 0.007	0.008	16:72:12
Chicken: smooth muscle, stomach	3	0.051 ± 0.005	0.008 ± 0.002	0.029 ± 0.002	0.014	16:57:27
Liver: guinea pig	4	0.094 ± 0.010	0.017 ± 0.006	0.039 ± 0.004	0.038	18:42:40
Kidney: rabbit	3	0.077 ± 0.003	0.015 ± 0.004	0.041 ± 0.002	0.021	20:53:27
Brain: rabbit	2	0.043 ± 0.002	0.015 ± 0.001	0.013 ± 0.002	0.015	35:30:35
Thymus: calf	2	0.297 ± 0.001	0.047 ± 0.015	0.034 ± 0.002	0.216	16:11:73
Pancreas: calf	2	0.160 ± 0.020	0.020 ± 0.002	0.049 ± 0.015	0.091	12:31:57
Spleen: rabbit, calf	2	0.114	0.016 ± 0.007	0.038 ± 0.006	0.060	14:33:53
Embryo: guinea pig	1		0.008	0.019		

A differentiation of purine nitrogen is obtained by the separate determination of purine nucleotides on the one hand and nucleoside-bound and free purine on the other. In this method nucleotides are precipitated as uranium salts after protein and nucleoprotein have first been removed, so that protein-bound nucleotides are presumably not determined. Hydrolysis of the uranium precipitate, removal of

[6] According to Berenblum, Chain and Heatley (13) pure connective tissue, as exemplified by tendon of the rat tail, contains no significant amounts of nucleic acid phosphorus.

uranium, formation of the cuprous purine precipitate and determination of its nitrogen yield the nucleotide-nitrogen value, whereas the nucleoside-bound and free purine nitrogen is obtained after hydrolysis and cuprous precipitation of the filtrate remaining from the first uranium precipitation.

Table VII shows data assembled from a paper of Barrenscheen and Peham (7). In discussing their results the authors call attention to the ratio between non-nucleotide (nucleoside and free purine) and nucleotide nitrogen. In the malignant tissue forms investigated this ratio (b : c) ranges between 0.9 and 2.1 (average 1.4 ± 0.5) while the normal tissues (brain and thymus excepted) range from 0.22 to 0.44 (average 0.37 ± 0.07). The high ratio in thymus (1.4) is considered as an expression of the involution of this fetal organ, while in commenting on the high value of brain tissue (1.2) reference is made to the similarities in the carbohydrate metabolism of brain and cancer tissue. Adenylic acid and adenosine triphosphate are considered to be the main components of the nucleotide fractions and evidence is cited suggesting that, at least in some tumors, free purines rather than nucleosides predominate in the non-nucleotide fraction. Similar determinations were made by Davidson and Waymouth (39-41) and a table has been constructed from some of their data (Table VIII). Although the tissue types used differed from those of Barrenscheen and Peham (7) the evidence concerning the non-nucleotide-to-nucleotide-purine ratio is similar: the values for malignant tissue range from 0.45 to 2.1, averaging 1.0 ± 0.5, while in normal rat livers and chicken muscles the ratio ranges from 0.12 to 0.64, averaging 0.33 ± 0.07. In the comparison (41) of butter yellow hepatoma with normal, embryonic and regenerating rat liver the respective average ratios (from groups of 5 to 8 animals) were 0.59, 0.32, 0.41 and 0.31.

2.5.3. *Nucleic acids.*—The nature of the nucleic acids of cancer tissue was first investigated by Willheim and Stern (203, 185). They prepared nucleic acid according to Levene (122) from 5 cases of human cancer, and for comparison from normal calf thymus and normal human liver. The products resulting (about 0.1 per cent of the tissue weight) from the cancer tissues contained 8.3 to 11.9 per cent N and 7.3 to 10.7 per cent P, compared with values of 14.0, 14.6 per cent N and 8.3, 9.8 per cent P for the nucleic acids of non-malignant origin. The authors' claim of having discovered "a chemical correlate of the anomalous nuclear structure of cancer" was challenged by Klein and Beck (103). They obtained from 8 normal human livers crude nucleic acid preparations in yields of 0.7 to 1.0 per cent which had a nitrogen content of 7.7 to 11.5 per cent. Recrystallizations, with recoveries of 15 to 27

TABLE VIII: AMOUNTS OF PURINE SUBFRACTIONS IN TISSUES

From Davidson and Waymouth (39-41)

	No. of specimens	Purine N (% of wet tissue, with standard error)			Ratio of 3 forms of purine N (total=100), in order of increasing complexity	Reference
		Non-nucleotide purines (b)	Soluble nucleotides (c)	Nucleoprotein purines* (d)	(b):(c):(d)	
Chicken: chemically induced tumor	4	0.009 ± 0.001	0.020 ± 0.001	0.100 ± 0.003	7:16:77	39
Chicken: Rous sarcoma	12	0.007 ± 0.0004	0.010 ± 0.001	0.060 ± 0.006	9:13:78	39
Chicken: muscle (heart, breast, leg)	11	0.011 ± 0.001	0.034 ± 0.003	0.035	14:42:44	40
Rat: fibroadenoma, breast	1	0.006	0.007	0.045	10:12:78	39
Rat liver: butter yellow hepatoma	5	0.010 ± 0.001	0.017 ± 0.003	0.145 ± 0.007	6:10:84	41
Rat liver: normal	16	0.010 ± 0.001	0.031 ± 0.001	0.168 ± 0.003	5:15:80	41
Rat liver: embryonic	6	0.007 ± 0.0003	0.017 ± 0.001	0.197 ± 0.015	3: 8:89	41
Rat liver: regenerating	8	0.011 ± 0.001	0.036 ± 0.001	0.173 ± 0.004	5:16.79	41
Man: lymphosarcoma	1	0.019	0.009	0.081	17: 8:75	39
Man: hypernephroma	1	0.010	0.016	0.071	10:17:73	39
Man: carcinoma of colon, secondary growth	1	0.010	0.011	0.028	20:23:57	39
Man: fibrosarcoma, thigh	1	0.013	0.021	0.111	9:14:77	39
Man: carcinomatosis (omentum)	1	0.009	0.005	0.031	20:11:69	39

* Calculated from nucleoprotein P figures, assuming for nucleic acid the ratio of 4 P: 10 purine N.

per cent, raised the nitrogen values to 14.0 to 15.5 per cent. The corresponding figures obtained for crude yield, nitrogen value, yield on recrystallization and nitrogen value of the recrystallized product, were 0.7 to 1.0, 8.3 to 10.4, 24 to 41, and 14.1 to 15.2 in 3 cases of metastatic liver cancer. The desoxypentose color reaction of Feulgen was also found to be identical in the preparations of different origin. Similar results, indicating identity, were obtained by Vowles (195) on nucleic acids isolated from Jensen sarcoma and thymus tissue. Recently Brues and associates (28) isolated specimens of thymonucleic acid as well as ribonucleic acid from a transplantable hepatoma which, while impure, did not differ significantly in nitrogen or phosphorus content from parallel preparations of noncancerous origin.

While the original findings of Willheim and Stern appear discredited by the subsequent investigations none of these seem adequate to decide the question of identity or non-identity or give structural insight similar, e.g., to that resulting from the comparison of the ribonucleic acids of yeast and tobacco mosaic virus by Loring (129) and which suggests that these two nucleic acids do differ in composition. The need for more intimate investigation of the nucleic acids of cancer appears more urgent in the light of further recent evidence indicating that nucleic acids may be biologically and chemically highly differentiated substances; a preparation having all the characteristics of a desoxyribonucleic acid was found by Avery, MacLeod, and McCarthy (5) to be the agent responsible for the transformation of one type of pneumococcus into another.

2.5.4. *Desoxyribonucleic acid.*—Data concerning the quantitative role of desoxyribonucleic acid in malignancy were published by Masayama and Yokoyama (135). Their results, while apparently precise, are lacking in details such as number of animals used and exact analytical procedure (Dische method); they show for butter yellow hepatomas an average desoxyribonucleic acid content, calculated as phosphorus, of 0.21 per cent (dry weight) or 0.044 per cent (fresh weight) compared with 0.080 and 0.023 per cent respectively for control rat livers. Very similar figures (0.26, 0.051 and 0.079, 0.023 respectively) were recently obtained by Schneider (173, 174) on analogous material, but with a new procedure in which nucleic acids are separated from protein by extraction with hot trichloroacetic acid. Dounce (45), on the other hand, determined the desoxyribonucleic acid content (also by the Dische method) of isolated nuclei and found in hepatoma 31 values nearly 50 per cent below those of normal rat liver, and approximately "normal" values for Walker carcinosarcoma 256. The estimated average values were 12, 22, and 18 per cent of the dry weight of the nuclei. Stowell and Cooper (188) studied the desoxyribonucleic acid content of human epidermoid carcinomas on a histological basis with the aid of quantitative photometric measurements of the Feulgen stain. They found that in 7 cases the carcinoma tissue gave values averaging 10 and 15 per cent higher than adjoining normal epidermis, expressed on a per-cell and per-volume-of-tissue basis respectively. However, the values ranged between —18 and +31 per cent, and —4 and+38 per cent.

Davidson and Waymouth (41), in another approach, extracted nucleic acids from defatted tissue powders with 10 per cent sodium chloride, and determined desoxyribonucleic acid with Dische's diphenylamine reaction. Their approximate average results for normal, malignant (butter yellow), embryonic and regenerating rat liver tissue are, expressed as phosphorus and on a dry and fat-free basis, 0.10, 0.14, 0.58 and 0.12 per cent, or calculated from their data for the non-defatted condition, 0.07, 0.11, 0.31 and 0.09 per cent. With regard to the normal tissue these data harmonize with the concordant results of Masayama and Schneider mentioned above, but they are definitely at variance with regard to the extent of the increase in the desoxyribonucleic acid content of hepatoma.

2.5.5. *Ribonucleic acid.*—In the investigation of Davidson and Waymouth (41) ribonucleic acid was determined on the lanthanum salts of the nucleic acids by means of the orcinol reaction, with average results of approximately 0.61, 0.55, 1.20 and 0.62 per cent, of the dry fat-free weight, of ribonucleic acid phosphorus, for the 4 liver tissues studied, in the order named above. The work of Davidson and Waymouth shows in quantitative terms that in mammalian tissue the ribonucleoprotein content is of the same order of magnitude as, and tends to be higher than, the desoxyribonucleoprotein content. Similar, although rather lower, ribonucleic acid figures were obtained by Schneider (174) by means of the trichloroacetic acid procedure described earlier. Both Davidson and Waymouth, and Schneider find an increase of the desoxy- to ribonucleic acid ratio in rat hepatoma, compared with normal liver. The possible significance of this finding is not necessarily invalidated by the discovery of the opposite relation when mouse lung tumors were compared with normal lung tissue (174), because whole lung tissue is presumably not comparable histologically to pulmonary cancer tissue (79). In a variety of human cancer tissues desoxy- to ribo-protein ratios of 0.29 to 0.83 were found (39), but pending data on homo-

logous normal tissues their significance cannot be further evaluated. While the data of Davidson and Waymouth, and of Schneider, do not support the cytochemical observations of Caspersson and his coworkers (31) concerning an association between rapid malignant growth and high ribonucleotide concentration, it must be remembered that the results of chemical fractionation of a whole tissue mass may not be comparable with measurements made on individual cells in the peripheral, actively proliferating tumor area. Furthermore, unlike the cytochemical measurements, the purely chemical methods do not differentiate between cytoplasmic and nuclear material.

2.5.6. *Nucleoproteins.*—If there were a well defined analytical or preparative basis for the differentiation of "free" proteins and nucleoproteins, *i.e.* proteins bound, in the native state, to nucleic acids by irreversible or reversible linkages, data concerning the composition of the protein components of nucleoproteins would require separate treatment. However, since at present no clear dividing line exists, as pointed out by Schultz (179), Sevag and associates (180) and by Gulland and collaborators (80), amino acid analytical data on nucleic acid-containing protein preparations have been included in the general section on protein components (section 2.4.). It may be useful instead to review briefly the diverse methods that have been used in connection with cancer studies for the preparation of "nucleoproteins," and to compare some characteristics of the resulting products. Extractions from tissue have been conducted with alkaline or neutral salt solutions, with a combination of the two, or with water.

By extraction of fresh tissue with 0.1 *M* sodium carbonate, precipitation with acetic acid, and extraction with alcohol and ether, Lustig (132) obtained from normal and cancerous human lung tissue products of 15.2 to 17.2 per cent N and 0.4 to 0.9 per cent P (P : N atom per cent ratio 1.3 to 2.3, average 1.8) in unstated amounts. By extracting normal and cancerous rat liver tissue in the cold with 0.5 *M* potassium chloride and 0.03 *M* sodium bicarbonate, at pH 8, precipitation at pH 4.2, repeated solution in, and reprecipitation from, 0.5 *M* potassium chloride and 0.15 *M* sodium bicarbonate, and final removal of bound lipid (72) Greenstein and Jenrette (70-72) obtained nucleoprotein fractions (N = 15.5 to 16.0) with a P : N atom per cent ratio of 2.0 to 2.5, and in amounts corresponding to approximately 5 per cent of the total tissue protein.

Bolaffi (23) extracted tissue brei for 24 hours in the cold with 10 volumes of 10 per cent sodium chloride solution (pH 6 to 6.7). Protein precipitating on heating the extract was discarded, and the fraction precipitating from the filtrate on slight acidification (pH 3.9) was repeatedly reprecipitated from its solution in 0.1 *N* sodium hydroxide and finally copiously washed with water, alcohol and ether. Ten preparations thus obtained from liver, spleen, kidney and heart of horse, calf, rabbit and guinea pig gave average values of 16.2, 13.4 and 1.22 per cent respectively for total nitrogen, amino nitrogen after hydrolysis, and phosphorus, or a P : N atom per cent ratio of 3.5 (0.6 to 6.0). The tissue residues remaining from the sodium chloride extraction were extracted with 0.025 *N* sodium hydroxide (at pH 9.5), and the dissolved protein was precipitated and purified as in the sodium chloride extraction. The average analytical values (N, amino N and P) of these products were 15.7, 12.8, and 0.34 per cent, with an average P : N atom per cent ratio of 1.0. Mouse and human tumors likewise yielded products of higher phosphorus content by salt extraction (1.4 to 3.2 per cent) than by alkaline extraction (0.8 to 0.9 per cent), and in one case it was demonstrated that the same holds true if an alkaline extraction (with 0.03 *M* sodium bicarbonate, at pH 8.1, in this case) precedes the salt extraction, instead of following it. No yields are reported.

Similar extractions were conducted by Rondoni (166) on benzpyrene sarcoma of the rat and, as control tissue (of doubtful validity), whole skin (epithelium and subcutaneous tissue). Ten volumes of 10 per cent sodium chloride for 5 hours, followed by 5 volumes for 16 hours, and 2 similar extractions, all in the cold, with 0.06 *N* sodium hydroxide were used. The main results (reported in terms of concentrations in the combined extracts) are summarized in Table IX. Analysis of the individual values

TABLE IX: NITROGEN DISTRIBUTION AND PHOSPHORUS CONTENT OF TISSUE EXTRACTS

From Rondoni (166)

		Mgm. (with average deviation) per 100 cc.					
		NaCl extract			NaOH extract		
	Number of specimens	Non-protein N	Protein N	P	Non-protein N	Protein N	P
Sarcomas	12	12.2 ± 0.4	23 ± 5	4.7 ± 1.0	9.6 ± 0.4	29 ± 7	4.6 ± 0.7
Skin	5	16 ± 5	30 ± 5	4.6 ± 0.8	9 ± 5	21 ± 6	2.6 ± 0.4

reveals significant positive correlations between the phosphorus contents of the two kinds of extract in the case of the skin values, and between the non-protein nitrogen contents of the two extracts in the case of the sarcoma values. While there are definite suggestions of similar correlations in the sarcoma phosphorus and non-protein data on skin, there is little (in the case of the skin samples) or no (among the sarcomas) evidence of correlation in the protein contents of paired extracts. It would seem, therefore, that the large range covered by the individual values within each series partly reflects actual variations in the nature of the specimens, but that inadequate technic of extraction is a strong contributory factor. Non-protein nitrogen and phosphorus tend to be completely (or reproducibly) extracted while the erratic protein values indicate their incomplete extraction. A substantial part of the phosphorus is undoubtedly non-protein (nucleotide) phosphorus, and the protein is not identical with, although it may comprise, the high-phosphorus protein fractions obtained by Bensley (12) or Mirsky and Pollister (138) with 10 per cent or 1 to 2 *M* sodium chloride. Rondoni's two protein fractions are stated to contain approximately one-half of the total tissue protein. A notable difference between the two tissue types is evident only in the phosphorus content of the sodium hydroxide extracts.

Kelley (96) extracted minced rat sarcoma, carcinoma and thymus tissue with 2½ volumes of water for 2 to 3 days at 4° C. Precipitation of the filtered liquid at pH 4 to 4.5, washing of the precipitate with water, reprecipitation from dilute sodium hydroxide (pH 9 to 10) and washing with water, alcohol and ether gave average yields of dry nucleoprotein, of 3 per cent of the fresh tissue weight of the cancer tissues, and of 7 per cent of the thymus tissue. Four separate preparations of each tissue type were analyzed, with remarkably constant results for each. Table X shows the average results with average deviations. The isoelectric points were determined electrophoretically. While there is a striking negative correlation between phosphorus content and isoelectric point, lack of a consistent relationship between sulfur and phosphorus seems to reflect individuality in the amino acid compositions. The author further concludes, on the basis of quantitative staining experiments, that the isolated nucleoproteins are "very similar in their nucleic acid-to-protein ratio to the nucleoproteins as they exist in the nucleus" of the corresponding alcohol-fixed tissue. This conclusion rests on a notable similarity in relative dye retention, at constant pH, of the three tissue types and the corresponding nucleoproteins, as shown in the following tabulation:

	Sarcoma 1	Carcinoma 256	Thymus
Tissue, dye retained at pH 3.5	100	132	168
Nucleoprotein, dye retained at pH 3.5	100	132	150

Additional evidence is contained in relative measurements at different buffer acidities (Table XI). However, the authors' conclusion that the relative phosphorus content of the isolated protein preparations also is proportional to the relative dye retention values of tissue and protein, is based on an erroneous mode of calculation.[7]

TABLE X: ANALYSIS OF NUCLEOPROTEIN PREPARATIONS
From Kelley (96)

	Atom per cent ratio					Isoelectric Point, pH
	N*	P*	S*	P : N	S : N	
Philadelphia No. 1 sarcoma	16.29 ± 0.10	1.06 ± 0.01.	1.06 ± 0.03	2.9 ± 0.1	2.8 ± 0.1	4.65
Walker No. 256 carcinoma	16.18 ± 0.08	1.65 ± 0.03	1.16 ± 0.13	4.6 ± 0.1	3.1 ± 0.4	4.4
Rat thymus	17.05 ± 0.14	2.89 ± 0.10	0.68 ± 0.12	7.7 ± 0.3	1.8 ± 0.3	4.25

* Average results in per cent, with average deviations (4 preparations each).

TABLE XI: COMPARISON OF DYE RETENTION OF TISSUES AND NUCLEOPROTEIN FRACTIONS
From Kelley (96)

	Increase in dye taken up due to increase in pH, expressed in per cent of amount of dye held at next lower pH.				
pH	2.8	3.2	3.55	3.9	4.3
No. 1 Rat sarcoma, nucleoprotein*				15	25
No. 1 Rat sarcoma, tissue†				16	24
No. 256 Rat carcinoma, nucleoprotein*			24	20	
No. 256 Rat carcinoma, tissue†			19	18	
Rat Thymus, nucleoprotein*		24	14		
Rat Thymus, tissue†		22	10		

* Unit: weight.
† Unit: area of tissue sections 7 microns thick.

[7] As shown by the phosphorus figures in the condensed tabulations given above, the nucleoprotein obtained from Walker No. 256 carcinoma contains 56 per cent, and that from thymus 173 per cent, more nucleic acid (based on the phosphorus values) than the nucleoprotein extracted from Philadelphia No. 1 sarcoma; and not, as the author states, 36 and 64 per cent, respectively.

Similar aqueous extractions have been used by Greenstein (69, 76, 77) and by Brues and associates (28). By one (69) or two (76, 77) hours' extraction of ground liver and hepatoma tissue with 3 volumes of water in the cold, Greenstein obtained solutions containing unstated amounts (perhaps one half) of the total tissue proteins. These extracts have a relatively high S : N atom per cent ratio (about 6). Phosphorus figures are not available. Brues (28) disintegrated liver or hepatoma tissue in the Waring blendor and extracted it with 5 volumes of water by stirring for 2 hours at 0° to 4° C. Trichloroacetic acid precipitation and lipid extraction yielded a protein preparation of 0.56 per cent P, in a yield of approximately 15 per cent of the fresh tissue. On the other hand, a precipitate obtained from the aqueous extract with 0.4 per cent calcium chloride solution contained one-half to two-thirds of the protein but 93 per cent of the phosphorus.

2.6. Metabolic Differentiation of Proteins

Ordinary chemical analysis—the measurement of the effect of well defined chemical reagents—is a tool of limited reach in the differentiation of proteins. Variations in the nature of proteins which as yet cannot be described in chemical terms, may in many cases be disclosed by interactions of a biological kind. The great potentialities of enzyme chemistry as an analytical instrument remain to be developed, and in regard to cancer proteins, there are few investigations based on an enzymatic analytical approach that deserve attention.

2.6.1. *Tissue digestibility.*—It has been asserted for a long time (for literature see references 82, 117, 118, 119) that in contrast to normal tissue, cancerous tissue is readily digested by trypsin but very little by pepsin. However, the actual experimental evidence appears quite inadequate to support such a conclusion in regard to homologous tissue pairs. Kögl in his much disputed studies on the presence of the unnatural stereoisomers of amino acids in cancer protein, and especially of glutamic acid (see section 2.4.5.) postulated that the presence of *d*-amino acids in the peptide chains should result in increased resistance to the normal digestive proteolytic enzyme systems. In testing this hypothesis (108) he fed dogs boiled Brown-Pearce carcinoma tissue, and for comparison boiled beef and boiled rice. From the feces of these dogs, protein fractions were isolated by saline and alkaline extractions; and from the urine, amino acids and peptides by mercury salt precipitation. Glutamic acid isolated from the feces contained, in 2 experiments, 53 and 59 per cent of the *d*-form after feeding of the tumor tissue, and 2 or 3 per cent after the feeding of rice or beef, while glutamic acid from urine showed a content of 17 and 19 per cent of the unnatural isomer after tumor feeding, and 0 to 2 per cent after rice or beef feeding. These apparently well documented observations have not been re-examined. However, Rodewald and Klein (164) upon feeding cancerous tissue, of either human or mouse origin, to healthy mice observed marked pathological changes in the livers of the animals, such as edema, cellular dissociation and infiltration of erythrocytes and leukocytes. No such effects could be produced by feeding a variety of other tissues, and while the liver changes were most pronounced in animals autopsied within 14 hours after ingestion of the cancer tissue, histological study after 4 to 6 weeks still showed evidence of regeneration following liver damage. According to the authors, *d*-amino acids are not responsible for these effects.

2.6.2. *Enzyme proteins.*—Proteinous substances found in the urine of cancer bearers and characterized primarily by their capacity to cause termination of pregnancy in experimental animals, have been described by Klar (100, 101) and Ely (53). They are briefly discussed in the section on excretory products (section 4.). Their presence was also demonstrated in primary and secondary carcinomas of various organs and in some normal tissues.

The enzymatic properties of cancer tissue have so far been studied primarily by means of quantitative assay for various enzyme systems defined in terms of the reactions they are capable of promoting. This rapidly developing field, accessible through recent reviews (192, 74), is outside the scope of the present study. However, as a matter of protein chemistry in the narrow sense, the problem of qualitative enzyme changes may be briefly considered. To what extent can the enzymatic behavior of malignant tissue be attributed to changed chemical properties of enzyme molecules rather than to changes in the amounts of "normal" enzymes present? A good deal of evidence has been developed in recent years in support of the concept that active enzymes consist of dissociable combinations of a high-molecular (protein) apoenzyme and a low-molecular coenzyme and that the specific properties of the combination are largely determined by the nature of the protein component. Specific properties of cancer enzymes may accordingly be viewed as expressions of protein specificities (43, 2). Orekhovich (148) found evidence for the qualitative specificity of a cancer enzyme system in the observation that, when cathepsins from normal liver tissue and from Jensen rat sarcoma were compared, the cancer

cathepsin was superior in its capacity to digest muscle proteins of the cancerous rat while on the basis of the ability to digest muscle protein of non-cancerous animals, the cancer cathepsin was the weaker of the two. A more exact approach to the problem of qualitative variations has been made by Blagoveshchenskii (20). Taking the temperature coefficient of the catalyzed reactions, and the energy of activation derived therefrom, as a specific quantitative characteristic of an enzyme system he observed that in Sinelnikov-Krichevski sarcoma and Brown-Pearce carcinoma the onset of tumor development is accompanied by abrupt decreases in the activation energies of proteolysis and hydrogen peroxide decomposition, followed by a steady increase until the animal dies. A preceding paper (19) reported that, apart from species variations in the activation energies of plant and animal catalases, the average energy of activation of autolytic proteolysis undergoes a gradual 13-fold increase during the life span of the guinea pig.

At least in one instance an attempt has been made to examine the question of the alteration of an enzymatic protein by its actual isolation. Kubowitz and Ott (115) isolated the protein component of a pyruvic acid hydrogenase from Jensen rat sarcoma and rat muscle tissue, and no difference between the two preparations could be discovered on the basis of elementary analysis, absorption spectrum, specific rotation, pH activity curves and in activation rates (see section 2.1.). Demonstration remains to be made, by similarly rigorous means, of a case of actual non-identity of an enzyme protein derived from different tissues, such as is implied in the findings of Blagoveshchenskii.

2.6.3. *Carcinogenic viruses.*—As protein components of cancer tissue, the primary identification of which rests on their biological action, the carcinogenic viruses must be mentioned. The evidence for the existence of virus-like entities in cancer tissue, their role as causative agents, and the ensuing generalized etiological theories cannot be discussed here (for recent reviews see references 147, 63), and it is sufficient for present purposes to list three separate lines of investigation which have disclosed the existence of virus-like substances of nucleoprotein character, associated with the propagation of malignant growths. Although the causative agent of the Rous chicken sarcoma has not been obtained as a pure chemical substance, a lipid-containing nucleoprotein fraction of an average molecular weight of 140,000,000 has been isolated, ribonucleic acid has been identified as a component of this material and it has been shown that its carcinogenic properties are lost when the nucleoprotein is decomposed into protein and nucleic acid or as a result of ultraviolet irradiation, the most effectively inactivating wave lengths being those maximally absorbed by nucleic acids (184, 37, 35). Cytologically the carcinogenic material of the chicken sarcoma has been tentatively identified with the cytoplasmic particles known as microsomes, and products of grossly similar characteristics have been separated by fractional centrifugation from chicken embryo and likewise from mouse embryo and mouse sarcoma (36). No report as to whether or not carcinogenic properties reside in the analogous mouse sarcoma fraction has as yet appeared. In the chicken sarcoma fraction the tumor-producing capacity is readily lost on standing in solution, even at low temperature (34), although the activity of the fresh material is very high; injection of 4×10^{-13} gm., *i.e.* 200 particles of molecular weight 140,000,000, produces a tumor in 1 to 2 weeks.

Papillomas of wild rabbits, extracts of which can give rise to malignant neoplasms, have yielded a protein which is monodisperse in the ultracentrifuge and electrophoretically homogeneous, and has an apparent molecular weight of 47,000,000. This substance is also highly active biologically, approximately 1,000,000 molecules representing the minimum carcinogenic dose, but unlike the chicken agent it retains its infectiousness for several months at 5° C., and contains desoxyribonucleic acid. An analogous protein fraction is not obtained from similar but non-infectious warts of the domestic rabbit (146, 189, 10, 9).

Lately, evidence has also been obtained indicative of the nucleoprotein character of the mouse milk factor capable of eliciting mammary cancer in susceptible animals. Purification has not yet been advanced as far as in the case of the chicken sarcoma and rabbit papilloma agents, but a minimum molecular weight of several millions has been estimated from ultracentrifugal measurements (92) and the agent has been shown to be inactivated at 60° C., to be stable between pH 5 and 10, and not to be inactivated by fat solvents (6). Some analytical data relating to these cancer-producing protein fractions have been summarized in Table XII.

The problem of possible specific antigenic properties of malignant tissue, while theoretically an integral part of the problem of the chemical nature of cancer proteins, belongs to the sphere of the immunologist and, therefore, no attempt is made to cover it in the present review. A chapter in the encyclopedic work of Stern and Willheim (186) provides an introduction into this rudimentary but important field.

TABLE XII. CARCINOGENIC NUCLEOPROTEINS

Product and reference	Yield, % of tissue	Kind of nucleic acid	Molecular weight	Nucleic acid content, %	Lipid content %	Whole product C %	H %	N %	S %	P %	Lipid-free product C %	H %	N %	P %	Isoelectric point pH
Chicken Tumor I Agent (36)	2.9*	Pentose	14×10^7	15-17	35	60.0	9.0	8.6		1.5	48.5	7.3	12.7	1.2	3.5
Chicken Embryo Fraction (36)	12.4*	"		15-17		59.5	8.7	8.2		2.1	49.9	7.0	13.8	1.2	
Mouse Embryo Fraction (36)	9.1*	"		15-17		54.6	8.5	8.5		2.1			14.3	1.4	
Spontaneous Mouse Sarcoma Fraction (36)	6.6*	"		15-17		56.3	8.9	8.0		1.5	49.3	6.8	14.5	1.2	
Induced Mouse Sarcoma Fraction (36)	7.2*	"		15-17		53.6	8.1	9.3		1.9	49.8	6.7	14.9	1.2	
Rabbit Papilloma Virus Fraction 9, 146)	0.001-0.1†	Desoxy-pentose	47×10^6	9	3‡	49.6	7.2	15.0	2.2	0.9					
Mouse Milk Factor (6)			4×10^6												4.8-5.1

* Per cent of dry tissue.
† Per cent of fresh tissue.
‡ Carbohydrate 6.5 per cent.

2.7. CANCER GROWTH AND PROTEIN COMPOSITION

As yet no comprehensive investigation attempting to correlate the progress of cancerous growth with possible changes in proteinous tissue components seems to exist. Only a few isolated data of interest from this point of view can be cited. Bolaffi (23) found that the atom per cent ratio of phosphorus to nitrogen in the nucleoprotein fraction extracted by 10 per cent sodium chloride from mouse adenocarcinoma was 3.7, 4.7 and 6.8, after 11, 20, and 26 days respectively of growth of the transplant. Masayama and Yokoyama (135) demonstrated that the thymonucleic acid content of the liver tissue (dry) of rats reached twice its normal value as early as 3 weeks after initiation of butter yellow feeding, and remained at approximately that level until 3 times the normal value was attained in the overt hepatomas developing after 20 and more weeks of feeding.

The data of Brown and Klauder (27) purporting to show a positive correlation between growth rate and sulfur content of mouse carcinomas have been mentioned earlier (section 2.4.2.) as have been the data of Drummond (46) displaying certain possible correlations between degree of malignancy and distribution of basic amino acids (section 2.4.1.). According to the previously (section 2.4.4.) cited two investigations of Edlbacher and Baumann (50, 51) on Jensen rat sarcoma, necrosis of the malignant tissue is reflected in a 30 per cent increase in tryptophane nitrogen, a 20 per cent decrease in histidine, a similar increase in lysine and a 5 per cent increase in arginine.

2.8. NUTRITION AND PROTEIN COMPOSITION

Relations between nutritional factors and cancer growth or cancer susceptibility form an important chapter of cancer research, and significant empirical observations have been made, such as the anti-cancer effect of caloric restriction, the inhibitory influence of deficiencies of essential amino acids, and certain relations between the nature of the dietary lipids and cancerous growth. The subject, broadly reviewed by Stern and Willheim (186) and more recently by Morris (140), concerns us here only from the point of view of the possible reflection of nutritional variations in the composition of the proteinous tissue elements.

The question was first examined within a limited range of quantitative terms by Drummond (47). Rats carrying sarcoma transplants were kept for approximately 1 month on 4 different diets, *viz.*, a normal diet of bread, oats, corn, greenstuffs and meat, an enzymatic meat hydrolysate, a meat hydrolysate prepared by boiling with sulfuric acid and, therefore, tryptophane-free, and a "monoamino acid fraction," prepared from the preceding material by removal of the phosphotungstic acid precipitate and excess phosphotungstic acid. The 3 artificial diets were supplemented with starch. Animals, approximately 5 in each group, and tumors grew well only on the normal diet, while on both tryptophane-free diets the animals as well as their tumors consistently lost

weight. Material for protein analyses was obtained by coagulation of minced tissue with slightly acid boiling water, precipitation of soluble protein by alcohol, and final extraction with ether. From each dietary group the tumors on the one hand and a combined representative sample of normal tissues (heart, lung, liver, spleen, kidney, skeletal muscle and gonads) on the other were treated separately. The summary on Table XIII shows that within the analytical criteria used and their presumed accuracy the dietary variations cause no striking change in either cancer or body tissues.

According to the nutritional theories of Freund (186) replacement of dietary animal fats by vegetable fats, i.e. replacement of saturated by unsaturated fatty acids, will prevent the formation of anti-carcinolytic or "cancer-cell-protecting" derivatives of the former. These ideas form the background for a study involving chemical analysis of cancer tissue, by Kellner and Lustig (97). Groups of mice, bearing carcinoma transplants, were fed a "normal" diet of raw milk and bread, and three different special diets, obtained by combinations of fat-free milk, carbohydrates and animal and vegetable fats. After 4 to 5 weeks on these diets crude protein samples were obtained by boiling tissue samples with acidified 0.7 *M* sodium sulfate solution. Protein nitrogen and non-protein nitrogen (in the filtrate) was determined, as well as protein sugar, i.e. reducing sugar liberated by leaving the tissue protein overnight with 2.5 N sodium hydroxide. The results may be summarized as in Table XIV. The only possible changes discernible in these data are increases in non-protein nitrogen and protein sugar in the tumor tissues grown on low protein diets. No correlated data on tumor growth or sizes are given.

In an investigation motivated by the theories relating malignant growth to sulfhydryl compounds Goerner and Goerner (65) determined by Tunnicliff's method of iodometric titration with nitroprusside as indicator the glutathione content of Flexner-Jobling carcinoma and rat liver under different regimens. The results, summarized in Table XV, show that a low cystine diet gives rise to a

TABLE XIII: DIET AND COMPOSITION OF RAT TISSUE
From Drummond (47)

	Total N %		Amid N %		Humin N %		Monoamino acid N %		Non-amino acid N %		Diamino acid N %	
Diet	C*	O†	C	O	C	O	C	O	C	O	C	O
Normal	13.8	12.8	5.3	6.3	3.2	4.6	50.8	50.6	5.4	6.5	34.4	32.2
Enzymatic hydrolysate	13.6	12.6	4.4	5.5	3.6	5.7	51.8	55.9	6.7	4.8	32.4	31.0
Acid hydrolysate	13.8	12.8	4.7	4.8	4.3	4.9	52.7	51.9	6.1	4.6	31.7	32.2
Monoaminoacids	13.8	12.4	5.0	4.9	4.0	4.7	52.4	52.6	6.5	4.0	32.0	32.7

* C=Dry, defatted cancer tissue.
† O=Dry, defatted organ tissue.

TABLE XIV: DIET AND COMPOSITION OF MOUSE CANCER TISSUE
From Kellner and Lustig (97)

Protein	Content of diet in: Carbohydrate	Saturated fats	Unsaturated fats	Dry protein, % of fresh tissue*	Protein N %*	Non-protein N % of protein*	Protein sugar % of protein*
	"NORMAL"			20.6 ± 0.9	14.0 ± 1.4	7.0 ± 1.0	1.2 ± 0.3
Low	high	low	high	20.2 ± 0.8	12.9 ± 0.6	10.5 ± 0.9	3.2 ± 1.0
Low	high	high	low	22.1 ± 0.7	11.8 ± 0.9	9.5 ± 0.3	2.3 ± 0.3
High	high	high	high	22.1 ± 0.7	12.2 ± 1.0	8.0 ± 1.3	1.6 ± 0.3

* Average values with average deviations (2 to 5 individual determinations for each dietary group).

TABLE XV: DIET AND GLUTATHIONE CONTENT OF TISSUE
From Goerner and Goerner (65)

Diet	Number of animals	Time on diet, days*	Tumor weight, gm*.	Glutathione in carcinoma, %*	Glutathione in liver, %*
I. Normal†					0.185(0.133-0.245)‡
II. Normal†	11		6.7 ± 1.8	0.060 ± 0.039	0.181 ± 0.022§
III. Cystine deficient¶	13	18 ± 3	6.0 ± 2.6	0.039 ± 0.029	0.086 ± 0.028
IV. Cystine deficient plus 0.4% cystine			||	**	**

* Average values with average deviations.
† Bread, oats, vegetables, butter.
‡ Range of values.
§ Range is 0.138-0.231.
¶ 72 per cent cooked navy bean meal, 4 per cent salt mixture, 15 per cent butter and 9 per cent lard.
|| Not distinguishable from that in Diet III.
** Not distinguishable from values obtained on Diet II.

significant lowering of the glutathione level of the liver and apparently also in the carcinoma tissue itself. However, these chemical tissue changes appeared in no way to affect the growth rate of the tumors.

3. PROTEIN COMPONENTS OF NON-CANCEROUS ORGANS

3.1. Protein Components of Tissue

3.1.1. *Protein properties.*—Various authors have considered the question whether the existence of a cancerous growth is reflected in the composition of the morphologically uninvolved tissues of the host organism. In the previously (section 2.1.) discussed investigation of Wolff (205) there are included 3 instances of a comparison of malignant human liver tissue and corresponding cancer-free liver tissue. The results (in per cent of the sum of the three protein fractions determined) were:

	Liver cancer	Cancer-free liver tissue	Normal liver
Euglobulin	11, 10, 14	15, 13, 15	29, 29, 28
Pseudoglobulin	14, 14, 16	16, 17, 15	15, 19, 21
Albumin	75, 76, 70	69, 70, 70	56, 52, 51

These results show a close similarity in the press-juice proteins of non-cancerous and cancerous parts, especially when comparison is made with the values obtained on 3 normal livers.

Orekhovich (149) concluded that changes occur in the "stability" of tissue protein as a result of the presence of a growing cancer transplant, and that the susceptibility to transplantation is related to such protein stability. The experimental basis for this conception lies in measurements of the digestion of tissues by catheptic extracts of Jensen sarcoma or liver. For instance, glycerin extract of Jensen sarcoma tissue was found to liberate the following amounts of amino nitrogen (Van Slyke) during 24 hours at 37° C. and pH 4.2 from different kinds of tissue:

Muscle tissue from	Number of determinations	Amino nitrogen per gm. tissue (average and extremes), mgm.
Healthy rats	31	2.6 (2.0-3.3)
Sarcomatous rats	16	4.9 (4.0-6.3)
Transplantation resistant rats	9	1.5 (1.0-1.9)

Numerous, although less clear-cut, data are shown demonstrating that the digestibility of skin tissue by liver cathepsin decreases to two-thirds of the normal value during the growth of Jensen sarcoma, and to less than one-half as the result of the growth of a Krichevski-Sinelnikov sarcoma. The experimental evidence permits no conclusion as to whether or not these findings are pertinent to the viewpoint of this review.

According to the previously cited work (section 2.6.2.) of Blagoveshchenskii (20) qualitative alterations of enzymes, as determined by changes in the temperature coefficient of the catalyzed reactions, are not restricted to the cancerous tissue itself, but involve the whole organism; and attention may also be called to the finding of Kritsman and Konikova (114) that glutamic and aspartic acid obtained from tissue proteins of animals affected with malignant tumors are contaminated with substances that inhibit transamination.

3.1.2. *Sulfur compounds.*—Toyoda and collaborators (191) studied the sulfur content in the organs of normal rats and of rats bearing Fujinawa sarcoma. In terms of dry, ash-free tissue, the summarized results (average percentages and lowest and highest values; 5 to 8 animals in each group) were as follows:

	Liver	Muscle	Heart
Normal animals	0.9(0.7-1.1)	0.7(0.4-1.3)	1.2
Sarcoma-bearing animals	1.6(0.9-2.4)	1.4(0.9-2.1)	1.6

These data indicate definite increases of sulfur content in liver, muscle and heart tissue as a result of the presence of a malignant growth, while kidney and spleen tissue showed no abnormality. Greenstein (69) in his work on sulfhydryl groups, found no difference in the sulfhydryl-to-nitrogen ratio of aqueous liver extracts of hepatoma-bearing and normal rats. As a rough approximation it is possible to estimate that perhaps 80 to 90 per cent of the nitrogen in these extracts is protein nitrogen, representing in turn in the neighborhood of one-third of the total protein. Thus the findings of Toyoda and Greenstein are not necessarily irreconcilable.

Non-protein sulfhydryl, *i.e.* glutathione, has been investigated by Voegtlin and Thompson (190, 194), Goerner and Goerner (65) and Woodward (207, 208), and a comparison of these investigations reveals several points of interest. In the former two studies, iodometric titration with nitroprusside indicator (Tunnicliffe method) was used while in the latter Woodward's highly specific enzymatic (glyoxalase) method was employed. The results, including the normal liver values of Fujiwara and associates (62) obtained by a third method, viz., starch-iodometric determination of total reducing substances and subtraction of ascorbic acid determined by Tillman's reagent, are summarized in Table XVI. The average normal liver glutathione values are remarkably concordant in all investigations. In two of them the presence of cancerous growth, transplants of Flexner-Jobling carcinoma (194), Walker No. 256 carcinoma (208), rat sarcoma (194) and Philadelphia No. 1 sarcoma (208), is reflected in a lowered glutathione

TABLE XVI: GLUTATHIONE CONTENT* OF RAT LIVERS

Authors	Normal animals Number	Normal animals Average and range	Carcinoma bearers Number	Carcinoma bearers Average and range	Sarcoma bearers Number	Sarcoma bearers Average and range
Voegtlin and Thompson (194)	65	178	12	161 (135-180)	8	122 (89-166)
Goerner and Goerner (65)	12†	185 (135-245)	11	181 (133-231)		
Woodward (208)	9	172 (140-198)	7	155 (110-204)	5	134 (102-154)
Fujiwara *et al.* (62)	14	171 (125-234)				

* Mgm. per 100 gm. of fresh tissue.
† Approximately.

content of the liver, while in transplantable hepatoma itself glutathione is higher than in normal liver, at least according to the data of Fujiwara and associates (62) (see section 2.3.2.). Goerner and Goerner, however, although working with the same neoplasm as Voegtlin and Thompson, encountered no lowering of liver glutathione. Since in a parallel experiment, in which the rats were fed a special cystine-deficient diet (cf. section 2.8., and Table XV), the average liver glutathione level was less than one-half of normal, while tumor growth did not seem to be affected, depletion of liver glutathione as a result of tumor growth may depend on nutritional conditions. The 3 diets used are described as follows: corn and wheat meals, dried milk powder, inorganic salts and cod liver oil (194), corn, oats, bread and lettuce (208), and bread, oats, vegetables, butter (65). It seems noteworthy also that in Voegtlin and Thompson's animals bearing carcinoma or sarcoma there appears a marked negative correlation between relative tumor size (0.05 to 25 per cent of body weight) and the amount (in per cent of fresh weight) of liver glutathione, whereas no such correlation is evident in the data of Goerner and Goerner (tumor weight 4 to 11 gm.), nor in those of Woodward (tumor weight 2 to 13 per cent of body weight).

methods they found tryptophane nitrogen fractions approximately 10 and 30 per cent higher than normal in livers and kidneys, respectively, of the tumor-bearers while in muscle there was no change. Rosenbohm (168) found the tryptophane content of the liver tissue protein of 5 Jensen sarcoma-bearers 16 ± 11 per cent higher than that of 8 healthy rats, while no significant differences were found in brain, kidney, spleen and muscle. Rosenbohm's absolute values are substantially lower and, because of preliminary purification of tissue protein, presumably more accurate than those of Edlbacher and Baumann. The tryptophane problem has been more fully discussed in section 2.4.4.

Klein and Ziese (104) studied the arginine content of the dry defatted muscle tissue of normal and cancerous rats. According to their data the presence of sarcoma in rats results in a lowered arginine content, while carcinoma in mice has the opposite effect. Furthermore, free, water-soluble arginine was found increased in the muscle of the cancer-bearing rats (Table XVII). While the paper is amply documented the full reliability of the method used (1 hour hydrolysis with 1 *M* sulfuric acid at 200° C., followed by a modified Sakaguchi method) remains open to doubt because of inconsistencies in the re-

TABLE XVII: ARGININE CONTENT OF TISSUE

From Klein and Ziese (104)

Muscle tissue of	Number of determinations	Dry, defatted tissue, %: N	Arginine N (as % of total N): Total (average and range)	Arginine N (as % of total N): Free (average)
Rats, normal	9	14.1	11.7 (10.8-12.8)	0.37
Rats, cancer-bearing*	8	13.8	10.5 (10.0-11.0)	1.19
Mice, normal (and with small tumors)	3	12.85	8.8 (8.5- 9.0)	
Mice, bearing Ehrlich carcinoma	4	12.6	12.3 (11.8-12.8)	

* 4 Jensen sarcomas and 4 Flexner-Jobbing carcinomas.

3.1.3. *Amino acids.*—Two investigations are concerned with the tryptophane content of the organs of tumor-bearing animals. In the paper of Edlbacher and Baumann (51) tryptophane average values, expressed as per cent of total nitrogen, are given for groups of 6 Jensen sarcoma-bearing and 6 normal rats. On the basis of 2 different analytical

ported recoveries of arginine added to the tissue proteins.

By extraction of tissue with 1 per cent sodium chloride solution and acidification of the saline extract with acetic acid, Schenck (172) obtained a "nucleoproteid" fraction and, by heat coagulation of the resulting filtrate, an "albumin-globulin" fraction,

while the defatted insoluble residue was termed "tissue protein" fraction. He determined the basic amino acids by the methods of Kossel (112), tyrosine and cystine according to Folin and Marenzi (58, 58a), and tryptophane according to May and Rose (136), on liver and muscle tissue of normal and Jensen sarcoma-bearing rats, using the pooled material from several animals for each determination. Those of his results that are of possible significance for the present section have been combined in Table XVIII. Increased cystine and tyrosine levels (and their high values) in the liver "nucleoproteid" and corresponding decreases in the other liver fractions, as well as increased arginine coupled with decreased histidine in the liver "tissue protein" seem to be significant accompaniments of the malignant growth of liver pulp or turpentine, although the effects were far smaller than those associated with the presence of tumors. The individual experimental results are listed only in terms of the purine quotient, but the average values given for total nitrogen indicate that the increased quotient is not the result of increased purine nitrogen but rather of decreased total nitrogen which, in part at least, can be attributed to the cloudy swelling. The increased desoxyribonucleic acid (DNA) content which Masayama and Yokoyama (135) found (Table XIX) in the non-cancerous part of butter yellow hepatoma liver, would account to only a small extent for the relative increase in purine nitrogen as the total purine nitrogen figures (49) are of the order of 0.15 per cent of fresh weight.

TABLE XVIII: AMINO ACID CONTENT OF TISSUE PROTEIN FRACTIONS

From Schenck (172)

	Yield % of fresh tissue		Total N %		Per cent of total N: Basic amino acid N		Arginine N		Histidine N		Lysine N		By weight %: Cystine		Tyrosine		Tryptophane	
	NA	SA	NA	SA	NA	SA	NA	SA	NA	SA	NA	SA	NA	SA	NA	SA	NA	SA
Rat liver																		
Tissue protein	14.1	13.3	14.8	14.5	36.2	35.1	8.2	9.8	20.0	16.7	8.1	8.6	2.4	1.9	3.8	1.6	1.5	0.8
Nucleoproteid	1.7	2.2	16.4	16.7	35.0	34.6	6.6	7.0					4.3	6.5	4.4	6.9	2.1	1.8
Albumin and globulin	0.6	0.6	...	...	...	...							3.4	2.6	4.4	2.6	2.9	2.6
Rat muscle																		
Tissue protein	...	...	14.0	15.4	32.3	32.6	9.0	10.0	18.7	17.8	4.5	4.9	1.4	1.7	3.5	3.6	0.5	0.7

N A = Normal animals.
S A = Sarcoma-bearing animals

although the single figures given permit no true evaluation. The manifest increase in muscle arginine cannot be compared with the contrary finding of Klein and Ziese (104) because the latter used the whole tissue protein.

3.1.4. *Nucleic acid and other non-protein nitrogen.*—Edlbacher and Jucker (49) (see section 2.1.) determined the purine content of rat organs and found that the presence of Jensen rat sarcoma (inoculated under the skin of the back) is reflected in an increased purine nitrogen quotient (per cent of total nitrogen) of the animals' liver. Their figures are as follows:

Livers	Number	Purine quotient (with average deviation)
Normal rats	13	4.6 ± 0.2
Sarcoma-bearers	11	5.8 ± 0.4

The authors mention "cloudy swelling" in describing the livers of the cancer-bearers and consider necrotic nuclear disintegration in the tumor and accumulation of disintegration products in the liver as the cause of the increases observed in purine content. Increased liver purine quotients could be produced by artificial necrosis induced by injection

TABLE XIX: DESOXYRIBONUCLEIC ACID CONTENT OF TISSUE

From Masayama and Yokoyama (135)

	Average results: DNA* per cent of dry weight	DNA* per cent of fresh weight	Purine N of DNA* per cent of fresh weight
Rat liver, normal	0.787	0.222	0.020
Hepatoma rat liver,			
Non-cancerous part	0.410	0.330	0.029
Cancerous part	2.115	0.444	0.050

* Desoxyribonucleic acid.

Euler and Schmidt (54) noted a different effect of cancerous growth on surrounding tissue. In rats carrying intramuscularly implanted Jensen sarcomas they obtained the following results, suggesting to them purine depletion of the surrounding tissue by the cancer:

	Number	Averages, with average deviation: Purine *N*, %	Total *N*, %
Sarcoma	8	0.18 ± 0.02	2.6 ± 0.1
Normal muscle	5	0.07 ± 0.01	3.0 ± 0.4
Muscle surrounding sarcoma	2	0.055 ± 0.001	4.7 ± 0.4

Kotchneff (113) measured amino nitrogen and polypeptide nitrogen in trichloroacetic acid filtrates of rabbit tissues and studied changes produced in these entities as a result of the growth of malignant Brown-Pearce epithelioma in the animal. She found that cancerous growth was accompanied by increasing amino nitrogen and, to a lesser extent, increasing polypeptide nitrogen content of liver tissue. Even more pronounced increases were found in spleen, and similar or smaller increases in kidney, muscle and the neoplastic tissue itself. A condensation (average results of approximately 6 specimens each, with average deviations) of the data shown for liver is as follows:

Mgm. per 100 gm. liver	Before transplantation	Days after transplantation 10-15	20-25	30-35	40-53
Amino N	64±9	87±8	103±15	126±10	177±26
Polypeptide N	61±9	71± 5	78± 4	79± 4	91± 8

3.2. Protein Components of Blood

The number of investigations dealing with relations between cancer and proteins of the circulatory system is large, but few results of real significance have so far emerged in this field. However, the great strides recently made in the exploration of serum proteins (38, 153) are likely to provide more promising possibilities than were heretofore available for the definite characterization of the possibly subtle changes associated with the malignant condition. If "the number of components that may be identified in the plasma proteins. . . remains far greater than the number of fractions into which it has been convenient to separate" them (38) the difficulties of the past appear understandable and the possibilities of the future become apparent.

3.2.1. *Whole blood.*—The whole blood as a tissue has been studied by Toyoda, Kishi and Nakahara (191) who used rats bearing the Fujinawa sarcoma and normal rats. In terms of the dry residue they found, in 5 cases each, 1.3 (1.2 to 1.4) per cent sulfur in the blood of the sarcoma bearers, compared with normal values of 0.8 (0.5 to 1.1) per cent. In ash content the normal blood was found higher (2.1 per cent) than that of the sarcomatous animals (1.5 per cent). It seems that no other work exists, confirmatory of or conflicting with these surprising observations. Two independent investigations dealing with the blood of Rous sarcoma-bearing fowls (139, 48) found no significant difference from the normal phosphorus values of the whole blood, although in the serum (which contains approximately one-tenth of the whole phosphorus) the phosphorus content was found to have doubled after 4 and 23 days of sarcoma growth (139).

3.2.2. *Protein fractions.*—Numerous investigations show that on a statistical basis the presence of malignant growth both in man and animals is reflected in a lowered concentration of serum proteins (186, 142, 75). Since, however, the ranges of individual values for normal and cancerous sera are wide and overlapping, and since cancer is only one of a diversity of pathological states associated with a low serum protein level, the significance of this phenomenon is limited and the voluminous literature dealing with it need not be reviewed in detail. In-

Table XX: Fibrinogen and Serum Globulin in Human Cancer

				Normal cases			Cancer cases		
Year	Author	Reference	Method	Number of determinations	Fibrinogen, mgm. per 100 cc. plasma	Globulin: albumin ratio	Number of determinations	Fibrinogen, mgm. per 100 cc plasma	Globulin: albumin ratio
1918	Loebner	125	Refractive index	19		33 : 67	44		54 : 46
1920	Loeper and Tonnet	126	?			36 : 64			61 : 39
1924	Galehr	64	Refractive index			33 : 67	60		41 : 59
1928	Starlinger and Winands	183	Micro gravimetric	25	250	35 : 65	14	530	53 : 47
1929	Louros and Gaessler	130	" "				15	660	47 : 53
1932	Exton and Rose	57	Photoelectric turbidimetric			29 : 71	16	increased	increased
1934	Goldfeder	66	Tyrosin index, colorimetric	6	200	37 : 63	25	520	43 : 57
1935	Kopaczewski	111	Acetone precipitation			41 : 59*	16		65:35*
1935	Loicq	128	Gravimetric	5	294		5	714	
1936	Reding	158	?	7		29 : 71	16		53 : 47
1936	Reding	158	?	3	363		13	656	
1936	Franklin, Sanigar and Allen	59	Photometric, ultraviolet	20		47 : 53†	26		60:40†
1938	Munro	142	?	30		34 : 66	35		41 : 59

* Ratio of globulin plus myxoprotein (cf. this section) to albumin.
† Ratio of fibrinogen plus globulin to albumin.

quiry may be made as to the underlying and possibly more specific deviations in the quantitative distribution or qualitative nature of individual protein fractions. The literature in this respect reveals a number of observations, obtained on different materials and with different methods, but agreeing in the conclusion that cancer is associated with an increased level of blood fibrinogen and serum globulin. A brief digest of the average values obtained by different investigators, revealing their striking consistency, is shown in Table XX. It must be added, however, that all these data refer to man. In regard to animals, Loicq (128) found the fibrinogen values of guinea pigs inoculated with the liposarcoma of Murray and of normal controls very similar (622 and 300 mgm. per 100 cc. plasma) to the corresponding values obtained on humans (Table XX). On the other hand, Dyer and Roe (48) found no significant difference in the globulin-to-albumin ratio (57:43 and 55:45, respectively) between hens bearing Rous sarcoma No. 1 and normal controls. Furthermore, while the globulin averages are consistently higher in cancer, the individual values cover wide ranges. Of greater importance, in every case where pathological material other than cancer was included in the investigation (64, 183, 130, 57, 128, 158) similar quantitative shifts in fibrinogen and globulin content were encountered (in conditions like tuberculosis, kidney disorder, puerperal sepsis and miscellaneous purulent infections).

Since both a low total serum protein content and a high globulin-to-albumin ratio have consistenly been found in association with human cancer, the serum albumin level obviously is low. And just as there exist certain striking chemical and biological similarities between embryonal and malignant tissue, so both fetal and cancerous growth may be accompanied by similar shifts in the proteins of the circulatory system. Rinehart (162) in a study comprising several hundred determinations found that in human pregnancy, total serum proteins are lowered as a result of an 18 per cent (average) decrease in serum albumins, the globulin level remaining within the normal range. Pedersen (152, 153) recently has demonstrated the occurrence of a new, relatively low-molecular (molecular weight about 50,000), protein type, called fetuin, in fetal blood. No information concerning the possible significance of this observation for the cancer problem is yet available.

Kahn (94, 83, 84) pursuing the shift in the serum albumin level of cancer bearers found that the difference is most pronounced in the most soluble fraction, i.e. the protein not precipitable by saturation with ammonium sulfate. In human cancer, Brown-Pearce carcinoma of the rabbit, and likewise in obstructive jaundice and advanced pregnancy, the amount of this fraction ("Albumin A") was found to be decreased to between two-third and one-half of normal, and an even more drastic decrease, to 20 to 25 per cent of normal, was found when the most extreme fraction ("Albumin S," soluble in 41.5 per cent ammonium sulfate in serum diluted 250 times) was measured. The earlier interpretation relating this phenomenon to the reputed high "albumin" content (cf. section 2.1.) of malignant tissue, appears to have been discarded by the discoverer in favor of the view (84) that the shift in soluble albumin is related to the presence of certain lipid fractions. That serum lipids profoundly influence the ultracentrifugal sedimentation diagram and the albumin-to-globulin ratio derived therefrom has recently been shown by Pedersen (153).

A differentiation of serum proteins more detailed than that into globulins and albumins, and one perhaps more specifically significant in relation to cancer, was introduced by Piettre (154). In his "cold acetone" method, serum proteins precipitated by 2½ volumes of acetone and redissolved in water, on acidification to the point of maximal precipitation yield globulins. Filtration of the globulins leaves a solution which, upon addition of 1 volume of acetone, yields a precipitate the water-insoluble part of which is termed "myxoprotein" (from its mucilagenous consistency). Step-by-step additions of further volumes of acetone yield further, increasingly water-soluble, precipitates. Their water-insoluble fractions represent additional "myxoprotein" while the combined water-soluble parts are considered as the serum albumins. A condensed summary (Table XXI) of data obtained by this method by Piettre (154) and Kopaczewski (111) shows what appears to be a rise in "myxoprotein" associated with cancer which is more significant than the changes in albumin and globulin, and more pronounced than the rise in "myxoproteins" in other disorders (pneumonia, syphilis, diabetes, myxedema, nephritis and cardiac cases).

TABLE XXI: HUMAN SERUM PROTEIN VALUES ACCORDING TO THE METHOD OF PIETTRE (154, 111)

Cases	Number	Average percentages (with lowest and highest values) Albumin	Globulin	Myxoprotein
Normal		59	28	13
Miscellaneous pathological	13	53(35-64)	23(15-41)	24(4-38)
Miscellaneous cancer	16	35(14-54)	22(14-32)	43(28-56)

Other expressions of changes in blood proteins have been reported. Bierry (16, 17) speaks of the

presence of glycoproteins in the euglobulin fraction of cancer patients, reporting values of 114 to 266 mgm. of protein sugar per 100 cc. of venous plasma in 10 cancer cases, compared with 60 to 80 mgm. in normals. However, similar relationships are encountered in infectious diseases. Hinsberg and Merten (86) determined the increase in reducing power of whole serum caused by hydrolysis at 100° C. with 0.25 *M* sulfuric acid. Precipitation with mercuric acetate is said to eliminate non-sugar reducing substances. Their study, covering 200 sera, shows that high values of "protein-bound" (defined by the method mentioned) sugar are characteristic but not specific for cancer. However, they also showed that liberation of these substances varies greatly with regard to rate among different sera, and independently of the nature of the clinical syndrome, and is in almost all cases by no means complete after the standard period of hydrolysis (4 hours) used. Obviously these investigations leave the question of protein-bound blood sugar unsettled.

3.2.3 *Physical properties.*—Munro (142) demonstrated a profound difference between normal and cancerous human sera in terms of protective colloid properties. In the presence of 0.075 cc. of serum, 15 mgm. of quinine hydrochloride (as a coagulating electrolyte) and 50 mgm. of congo red per 10 cc., the following amounts of the dye remained in solution:

	Milligrams (average and range)	Percentage of cases below 0.150 mgm.
70 Normal sera	0.405 (0.100-2.00)	4
78 Cancer sera	0.093 (0.026-0.34)	90

Greenstein and Thompson (75) followed the changes occurring in the serum of rats as a result of the growth of Jensen sarcoma transplants, by measuring the colloid osmotic pressure. Their determinations, made in 2-day intervals on 3 rats each time, reveal the following trend:

Days after inoculation	0	5	12	19
Colloid osmotic pressure, mm. Hg, average	326	286	241	201

The net decrease of 38 per cent compares with an average decrease of 21 per cent in the quantity of serum protein which was determined on the same material.

3.2.4. *Amino acid content.*—As to chemical characterization of the individual serum protein fractions, there is the work of Bierich and Lang (15). They studied globulin (precipitated by 21.5 per cent sodium sulfate), albumin I (precipitated by 30.5 per cent sodium sulfate) and albumin II (the protein remaining in solution). In an investigation covering 32 miscellaneous human sera and 23 cancer sera no significant differences were found in biuret-forming groups nor in titratable acid groups of the 3 fractions. Tryptophane determinations gave the following results (averages in per cent, with "mean error"):

	Globulin	Albumin I	Albumin II
Non-cancerous	3.34 ± 0.05	1.05 ± 0.05	0.504 ± 0.010
Cancerous	3.46 ± 0.06	1.21 ± 0.05	0.435 ± 0.010

These figures suggest a significant lowering of the tryptophane content of the more soluble albumin fraction. It is interesting to note that the highly soluble "albumin A" fraction of Kahn, which is said to be decreased in cancer serum, contains no tryptophane (83), thus necessitating postulation of a more pronounced lowering of the tryptophane content in another part of the "albumin II" fraction in order to reconcile the two observations.

Rosenbohm (169) following up the observations of Bierich and Lang (15), which were made on human sera, by using the same experimental methods on rats found the following figures for the tryptophane content of albumin II:

15	Tumor-free rats	0.461 per cent
15	Rats with Jensen sarcoma	0.569 "
6	Rats with Walker carcinoma	0.511 "
4	Rats with Flexner carcinoma	0.594 "

The surprising observation that a change in tryptophane content occurs in opposite directions in rat and man may find its explanation in the operation of two simultaneously occurring changes suggested by the joint consideration of the findings of Hanke and Kahn (83) and of the Hamburg authors (15, 169). If among the more soluble serum proteins in cancer there are two fractions of lowered tryptophane content one of which is decreased in amount while the other is increased the net result could depend on the relative extent of these changes. The reported (83) high histidine content (7 per cent) of "albumin A" should be of value in further explorations of this situation. Rosenbohm (169) calls attention to the fact that the tumor-to-blood ratio is 10 to 30 times as large in the experimental cancer rat as it is in typical cases of human cancer.

3.2.5. *Non-protein nitrogen (incl. glutathione).*—Investigations concerned with the non-protein nitrogen of the blood and its subfractions are numerous, and discussion is limited to a few of those that in retrospect appear to contribute data of significance. Numerous observations show that elevated "polypeptide" nitrogen (*i.e.* of substances precipitated by tungstic or phosphotungstic acid but not by trichloroacetic acid) values are a characteristic

accompaniment of many cancer cases (81, 158, 120, 210, 39, 204), but it also seems evident (81, 158, 120, 210, 204) that this quantitative alteration is not specific for cancer but is found associated with various inflammatory conditions. In fact, investigations on selected human cases, guinea pigs (127) and dogs (124) have led to the conclusion that in cancer uncomplicated by ulceration and other secondary conditions, the blood polypeptide level is unchanged. The nature of the methods used leaves room to doubt the validity of some of these conclusions. The "double nitrogen" method of Hahn (81) appears to be the basis of most of the procedures used by subsequent investigators for the determination of polypeptide nitrogen. The latter is obtained as the difference of two experimental values; namely, the nitrogen contents of the trichloroacetic acid filtrate and the tungstic (or phosphotungstic) acid filtrate. As the difference is small (1 to 10 per cent of the experimental values) it is burdened with large systemic errors, and it must be assumed that the error is further increased to a large and variable extent in view of recent evidence (89) demonstrating that the presence of tungstates causes substantial deficits in ordinary Kjeldahl nitrogen determinations.

A recent investigation on normal and Rous sarcoma-bearing fowls by Davidson and Waymouth (39) disclosed significant changes in serum polypeptide nitrogen. In 11 normal controls the polypeptide nitrogen (in mgm. per 100 cc.) averaged 12.3, with only one value higher than 14; in 12 Rous sarcoma-bearers the average value was 18.5, with 3 cases below 14. These results are of interest because the polypeptide determination was direct (30), *i.e.* by determination of the nitrogen content of the phosphotungstic acid precipitate secured from the trichloroacetic acid filtrate, so that at least the major one of the two sources of error mentioned is avoided. They are further noteworthy when viewed in relation to another study. Rose and Dyer (165) found no significant change in the non-protein nitrogen of the blood of hens as a result of the presence of a Rous sarcoma, nor in uric acid and creatinine. While the data of Davidson and Waymouth are hardly at variance with those of Rose and Dyer as regards the absence of a decisive change in non-protein nitrogen (Table XXII) the results of their polypeptide determinations show that changes in this subfraction may not necessarily be reflected in the whole non-protein fraction.

Data obtained in a recent investigation on rats by Winzler and Burk (204) reveal the relations summarized in Table XXIII. In these figures the facts of special interest are that in the cancerous animals non-protein nitrogen (sulfosalicylic acid filtrate; similar figures were obtained by trichloroacetic acid) shows a definite increase; that the relative increase in the "polypeptide" subfraction(determined by the "double nitrogen" method, *i.e.* precipitations with sulfosalicylic and tungstic acid) is greater; and finally that the most pronounced increase occurs in the non-dialyzable ("proteose") fraction. Furthermore, in the cancer cases this non-dialyzable fraction largely escapes tungstic acid precipitation indicating that it differs not only in quantity but also in its chemical nature from the analogous normal fraction. The authors report a few preliminary analytical results (16 per cent N, 1.5 per cent P, 0.9 per cent S, 1.0 per cent cystine, 2.6 per cent methionine) for the non-dialyzable fraction isolated from normal blood, but comparable data for the analogous fractions obtained from cancerous animals, which would be of interest in view of the difference in precipitation behavior, are not given.

TABLE XXII: NON-PROTEIN NITROGEN IN BLOOD OF FOWL

	Normal				Sarcoma-bearing			
		Nonprotein N (mgm. per 100 cc.)				Nonprotein N (mgm. per 100 cc.)		
Reference	Number of animals	Average	Range	Av. deviation (% of average)	Number of animals	Average	Range	Av. deviation (% of average)
165	13	36	28-47	± 14	10	35	26-50	± 20
39	11	47	34-62	± 13	12	55	42-67	± 13

TABLE XXIII: NON-PROTEIN NITROGEN FRACTIONS OF RAT BLOOD

From Winzler and Burk (204)

	N* in sulfosalicylic acid filtrate			Additional N* precipitated by tungstic acid			Non-dialyzable N* in sulfosalicylic acid filtrate		
	Number of cases	Average	Range	Number of cases	Average	Range	Number of cases	Average	Range
Normal rats	9	45	38-51	3	3.8	3.5-4.5	6	3.8	3.0-4.5
Rats carrying hepatoma or sarcoma	12	64	53-83	6	13	7.5-18	6	32	17-42

* Mgm. per 100 cc. of blood.

The amino acid (more exactly free amino nitrogen) content of blood shows a rise in the presence of cancer but the same result can be due also to other conditions. Table XXIV represents a summary of some data obtained on humans.

One of these investigations (133) affords a comparison between humans, rats, and mice with regard to serum protein (by acid coagulation) and amino nitrogen (by formol titration of the deproteinized serum); it is summarized in Table XXV. Although in the animals the absolute amino nitrogen level appears to be independent of the cancerous process a substantial increase (about twice as large as in humans) in relative amino nitrogen concentration, shown in columns (d) and (h) of Table XXV, is evident.

There is thus considerable concordant evidence as to the association of quantitative shifts in polypeptide and amino nitrogen with cancer; but there is little information concerning the chemical identity of the compounds involved. The previously mentioned results of Winzler and Burk (204) indicate profound changes in the qualitative nature of the polypeptide fraction; and with regard to amino acids cancer has been shown to be associated with a significant lowering of the blood arginine level (160). In this study arginine was determined by the Sakaguchi method on the trichloroacetic acid filtrate of oxalated whole blood. The results, expressed in mgm. arginine per 100 cc., are as follows:

Male rabbits, normal	5.4 ± 0.4	(8 cases)
Male rabbit, with Brown-Pearce carcinoma	3.1 ± 0.7	(5 ")
Male rats, normal	5.2 ± 0.2	(6 ")
Male rats, with Jensen sarcoma	3.8 ± 0.4	(6 ")

Additional experiments indicated that a lower arginine level is also associated with normal growth processes such as pregnancy or hormonally induced uterine and ovarian proliferation:

Female rabbits, normal	5.7 ± 0.7	(11 cases)
Female rabbits, pregnant	2.5 ± 0.4	(2 ")
Female rabbits, after delivery	6.1	(1 case)
Female rabbits, after injections of gonadotropic hormone	3.6 ± 0.1	(4 cases)
Female rabbits, preceding 4 cases after ovariectomy	5.3 ± 0.4	(4 ")

In the ovariectomized animals hormone injections left the arginine level unchanged. Despite their nonspecificity these clear-cut results seem to deserve attention from the point of view of cancer metabolism because of the correlated findings of high arginine concentrations in malignant tissue (see section 2.4.). The arginine values account for only approximately 2 per cent of the previously cited amino nitrogen figures, and other amino acid determinations appear not to have been made, at least in connection with cancer studies.

TABLE XXIV: AMINO ACID CONTENT OF HUMAN BLOOD

	Non-cancer cases			Cancer cases			
Method	Number	Average*	Range*	Number	Average*	Range*	Reference
Formol titration†	8	14.4	11.6-17.2	9	17.8	10.6-24.0	133
Folin colorimetric‡	11	6.3	5.2-7.8	21	8.5	6.8-10.4	158
" "		8.2§	6.7-10.1§				158
" "	10	8.3	7.0- 9.6	19	11.5	9.4-14.2	210
" "	5¶	9.5	8.5-10.6				210

* Mgm. N per 100 cc.
† Determinations on serum.
‡ Determinations on hemolyzed whole blood.
§ Post-operative clinical cases.
¶ Cases of stomach ulcer with gastritis.

TABLE XXV: SERUM PROTEIN AND AMINO NITROGEN IN CANCER
According to Malowan (133)

NON-CANCER CASES

	Number	Protein N*	Amino N*	Ratio $\frac{100(c)}{(b)}$
	(a)	(b)	(c)	(d)
Humans	8	1440 ± 60 (1280-1570)	14.4 ± 1.5 (11.6-17.2)	1.00
Rats	7	1220 ± 180 (1020-1440)	28.0 ± 2.4 (20.0-32.6)	2.30
Mice	2	1155 ± 65 (1090,1220)	34.8 ± 2.9 (31.9-37.6)	3.00

CANCER CASES

	Number	Protein N*	Amino N*	Ratio $\frac{100(g)}{(f)}$
	(e)	(f)	(g)	(h)
Humans	9	1390 ± 80 (1200-1520)	17.8 ± 3.3 (10.6-24.0)	1.28
Rats	9†	770 ± 140 (540- 930)	28.1 ± 3.3 (17.5-32.8)	3.65
Mice	2‡	675 ± 15 (660, 690)	31.0 ± 3.4 (27.6,34.3)	4.60

* Average, average deviation and range of values, in mg. N per 100 cc. of blood.
† Rats with Jensen sarcoma.
‡ Mice with Ehrlich carcinoma.

Discussion of blood glutathione in relation to cancer will be limited to only a few important investigations (176, 177, 206, 150). The method used in all cases was the iodometric procedure of Woodward and Fry (206) which, according to Woodward (207), in the case of blood gives close agreement with the more specific enzymatic method (207, 44) although in tissue the iodometric method may overestimate the actual glutathione content by several hundred per cent, due to the presence of ascorbic acid and other reducing substances. The net results of these investigations as regards whole blood, have been summarized in Table XXVI.

While the presence of cancer finds no reflection in these measurements further differential analysis led to different conclusions. Schoonover (177) determined the ratio of oxidized to reduced glutathione separately for plasma and erythrocytes and found in a series of 21 cancer patients and 13 normal cases that, while either ratio covers wide and overlapping ranges, a given erythrocyte ratio is consistently associated with a higher plasma ratio in cancer than in normals. This relation finds numerical expression in the quotient $\frac{GSSG}{GSH}$ (plasma) : $\frac{GSSG}{GSH}$ (erythrocytes); in the series mentioned earlier its highest value among the normals was 7.1 and its lowest value in cancer 7.2. However, no diseases other than cancer were investigated. This approach was resumed and expanded by Osterberg and associates (150), and the results of both investigations, as regards the quotient of Schoonover just defined are summarized in Table XXVII.

Displacement of the quotient in cancer is confirmed by the second investigation and despite overlapping ranges seems to be highly characteristic for cancer and, in addition, liver disease. Further investigation would seem to be warranted because the values of Osterberg and associates for oxidized glutathione are definitely at variance with those of their predecessors (see Table XXVI) so that differences of technic may be involved even though the same method was used. Furthermore, there is no certainty that Woodward's statement (207) that "with blood, there is quite close agreement (with the enzymatic method) indicating that with the iodate titration procedure for blood only glutathione is estimated" holds true in all cases, and the enzymatic method (178, 44) might reveal the actual relations more clearly, especially since by the latter method "no oxidized glutathione was found to occur natur-

TABLE XXVI: GLUTATHIONE CONTENT OF HUMAN BLOOD

Normals				Cancer patients				
Number	Total*	Reduced*	Oxidized* (by difference)	Number	Total*	Reduced*	Oxidized* (by difference)	Reference
10	42 (37-47)	35 (29-41)	7	5	37 (32-42)	30 (26-36)	7	206
12	41 (31-53)	36 (23-44)	5	42	40 (22-55)	34 (18-48)	6	176
37		35 (20-47)		37		29 (11-42)		150
38	56	37	19	32	47	32	15	150

* Mgm. glutathione per 100 cc. of blood; average and range.

TABLE XXVII: DISTRIBUTION OF OXIDIZED AND REDUCED GLUTATHIONE IN HUMAN PLASMA AND ERYTHROCYTES

Classification	Number	Schoonover quotient* (average and range)	Cases above critical value,† %	Reference
Miscellaneous cancer cases	21	17.8 (7.2-36.2)	100	177
Normal subjects	13	4.2 (1.6- 7.1)	0	177
Normal subjects	38	5.6 (2.6- 9.8)	5	150
Miscellaneous pathological conditions	88	5.9 (0.8-21.0)	20	150
Benign tumors	10	7.3 (3.1-18.1)	30	150
Non-malignant liver disease	7	10.5 (6.3-17.4)	71	150
Carcinoma of colon	43	12.5 (3.9-46.0)	81	150

* $\frac{GSSG}{GSH}$ (plasma) : $\frac{GSSG}{GSH}$ (erythrocytes).

† Empirically selected by the reviewer: 7.1 in Schoonover's data, 7.4 in those of Osterberg *et al.*

ally in blood or tissues" (44). Also, it would seem to be of value to consider the possible relation of these determinations to the polarographic abnormalities observed in cancer sera by Brideka (26) and others (197, 199), and to the findings of Yampolskaya (210) on the erythrocyte-plasma quotients for amino and polypeptide nitrogen. The latter's data, relating to 8 healthy persons and 21 clinical cases, reveal that the ratio of polypeptide to amino nitrogen in plasma tends to be increased in cancer, just as the ratio of oxidized to reduced glutathione in plasma tends to be increased (177), and that a given polypeptide-to-amino nitrogen ratio in the erythrocytes tends to be associated with a higher ratio of the same two characteristics in plasma. This relation can be adequately expressed by the quotient $\frac{RNHCOR}{RNH_2}$(plasma) : $\frac{RNHCOR}{RNH_2}$(erythrocytes), which is in form analogous to the above mentioned glutathione quotient. Selecting empirically from Yampolskaya's data 1.2 as the critical value of the polypeptide quotient one finds that it is exceeded in 1 of 8 normals (13 per cent), 14 of 16 cancer cases (88 per cent) and 3 of 5 (60 per cent) cases of stomach ulcer with gastritis, the latter serving as non-cancerous pathological controls. No implication can be attached to this merely formal analogy but one may note that in normal erythrocytes the polypeptide nitrogen of glutathione calculated from the total glutathione figures of Schoonover (177) accounts for 75 per cent of the total polypeptide nitrogen of Yampolskaya (210), which is determined according to Hiller and Van Slyke (85), i.e. by the increase in the amino nitrogen content of the trichloroacetic acid filtrate caused by acid hydrolysis. Simultaneous investigation of peptide and glutathione variables on a wide range of clinical material might be illuminating. In connection with possible changes in the erythrocytes recent observations as to clear-cut differences in the physicochemical properties of embryonal, infant and adult hemoglobin (3, 209) may deserve attention.

4. PROTEIN COMPONENTS OF EXCRETORY PRODUCTS

The principal observations to be recorded under this heading deal with the occurrence in the urine of cancer-bearers, of substances which upon injection cause termination of pregnancy in experimental animals. Elsasser and Wallace (52) observed that intravenous injection into a pregnant rabbit of 20 cc. of the urine of a cancerous individual caused abortion, a reaction not produced by human pregnancy urine. Similar observations were made independently by Klar (100, 101) who found the active principle in the urine of all of 25 human cancer cases investigated as well as in 9, mostly inflammatory, conditions among 33 nonmalignant clinical cases. The active principle was precipitable with trichloroacetic acid, showed positive biuret and ninhydrin reactions, contained sulfur and phosphorus, but yielded negative tests for tyrosine, histidine and tryptophane. The substance, insoluble between pH 3.5 and 6.0, was also found in carcinomatous tissue and certain normal organs "bound to the albumin fraction of tissues." Presumably the same agent was dealt with by Ely (53). His concentrate, prepared from cancerous and, with lower activity, from normal human urine, caused abortion or fetal resorption, depending on the animal used; the qualitative characteristics reported (positive tyrosine, but negative biuret, ninhydrin and tryptophane tests) differ from those of Klar.

A report[8] by Muniz (141) speaks of the disappearance of conjugated sulfur compounds from the urine in cancerous and precancerous conditions.

5. CONCLUSION

In a paper written forty years ago one reads that "the great need in the study of malignant growth is additional facts; there are plenty of theories" (11). Since that time, the body of both fact and theory has grown, but a solid framework of the chemistry of cancer likely to accommodate the physiological phenomena has not yet arisen. The data here reviewed hardly justify an attempt to summarize factual conclusions derivable from them because of the great disparity between problems raised and results established.

As a phenomenon of abnormal growth, cancer involves abnormal protein synthesis, but abnormal protein synthesis may either mean the synthesis of normal proteins at the wrong time, and in wrong places and amounts, or it may mean the synthesis of specifically abnormal proteins. The present literature contributes little toward a decision between these basic alternatives, and its study seems to indicate that the past experimental approaches have largely served to reveal the technical complexities of the problem. Some of these difficulties have already been touched upon in the treatment of the literature; their more important aspects may be labeled briefly as the problem of (a) homologous tissue, (b) homogeneous tissue, (c) deterioration of

[8] Evaluation of its experimental basis was not possible because of the omission of two pages (p. 426 follows on the back of p. 423) in the original printing.

tissue, (d) distintegration of tissue, (e) the structural complexity of cells, and (f) the dynamics of metabolism.

(a) If one concedes that each specialized cell type has its own chemical pattern and that different types of malignant cells originate from different types of normal tissue cells then it is clear that the chemical changes specifically associated with the malignant cell modification cannot be brought to light unless one compares a particular malignant tissue with its specific tissue of origin (homologous tissue). Comparisons of cancer of the mammary glands with whole breast tissue or of cancer of the alveoli of the lung with whole pulmonary tissue, and so forth, then appear of doubtful value. (b) The problem of homogeneous tissue may be characterized by reference to the observation made in a microscopic study of human neoplasms (170, 171) that in the average only 56 per cent of the tissue cell area consisted of malignant cells. Some human and rat cancers were shown to be made up to 95 and 100 per cent of malignant cells, and for critical chemical studies material of an established degree of histological uniformity would have obvious advantages. (c) Depending on the type of tissue used (liver tissue is distinguished by being subject to very rapid autolytic changes) and the chemical entities considered (nucleosides, nucleotides, and purines are known to undergo rapid alterations) autolytic postmortem deterioration imposes different degrees of limitation upon the experimental approach. However, experimental possibilities have been greatly extended by modern low-temperature equipment and technics. (d) A cell is a multiphase system in which proteins serve structural and functional ends, and in which structural differentiation is essential to functional complexity and order. Because the structural barriers of the cell act as chemical barriers an over-all chemical analysis of tissue cells depends on adequate destruction of cellular structure prior to extraction, and absence of this difficulty in the investigation of plasma proteins may largely explain why today the field of tissue proteins is essentially *terra incognita* by comparison with the physically and chemically well explored sphere of blood proteins. As far as liberation of soluble cellular proteins and lipids is concerned modern devices such as the Waring blendor seem to be far superior to previous tools of tissue destruction. (e) Because of the structural organization of the cell an over-all analysis obviously affords limited insight into the significance of changes in chemical composition. It would, for instance, be of fundamental importance to know if an observed difference in the composition of cellular proteins is an expression of different nucleus-to-cytoplasm ratios or of the presence of different protein entities in the cytoplasm (or the nucleus). This type of problem may be met by either accompanying chemical analysis with cytological analysis or by separation of cytological components (nucleus, chromosomes, mitochondria, microsomes, etc.) preliminary to chemical analysis. (f) In the analysis of such actively growing tissue as cancerous tissue, it may be very important to give consideration to the fact that living tissue is differentiated not only in the dimensions of space but also in that of time. Continuous metabolism means continuous chemical change; but, beyond that, there is evidence that the process of active cell division may be subject to long-wave (wave lengths of the order of hours) rhythms (159). The period of the mitotic cycle at which the tissue is killed preliminary to analysis may therefore be an important determinant of the resulting analytical picture.

Another class of difficulties comprising those which are of a more general analytical nature and not peculiar to tissue needs no elaboration. Recent developments such as the methods of analysis by isotope dilution and microbiological assay have greatly increased the ranges of analytical precision and specificity for many protein components.

Consideration of the whole compass of biological and chemical problems involved in the question of tissue proteins, together with the unrealized potentialities of modern physical means of protein separation and characterization, and in its light a reappraisal of the efforts here surveyed and their meager results, seems to constitute a compelling argument for the urgency of basic and cooperative research to the cancer problem.

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On the Fate of Carcinogenic Hydrocarbons in the Animal Body

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The study of the fate of carcinogenic substances in the body was first taken up in 1934-36 by Chalmers and Peacock (8, 17), Berenblum and Kendal (1, 2), Hieger (11), Lorenz and Shear (14) and others. Extension of knowledge in this field is necessary for further progress in studying the mode of action of carcinogenic substances and the pathogenesis of malignant tumors induced by these chemical factors. Moreover, if the carcinogenic hydrocarbons do not greatly differ in their structure and physicochemical properties from the supposed endogenous carcinogenic substances, the study of these questions may yield material for drawing certain analogies with respect to the latter. In this connection it is of interest to study the fate of carcinogenic substances not only in a healthy organism, but also in one affected with pathologic lesions.

The investigations we started in 1940 on some of these problems together with my assistants B. Vigdorovich, I. Plindov, M. Zalesskaya and A. Friedman were interrupted by the war in June 1941 and the spectrograph we had been using was entirely ruined. Data which we had obtained before this interruption are presented in this paper.

MATERIALS AND METHOD

The investigations were carried out on over 100 mice, 60 rats, 50 rabbits, 15 dogs and 1 cat. The carcinogenic hydrocarbons used were for the most part 3,4-benzpyrene, to a lesser extent 20-methylcholanthrene and 1,2,5,6-dibenzanthracene. To get a general notion of the hydrocarbon present in bile, urine, cerebrospinal fluid or at the site of subcutaneous injection visual examination of the fluorescence of these materials in a filtered ultraviolet beam was made. To discover the presence of hydrocarbons in the viscera, in blood and other fluids we usually prepared benzene extracts according to Berenblum and Kendal's second method (2). The fluorescence spectrum of these extracts, reduced to the same volume, was photographed by means of the spectrograph. Most of the investigations were made with a spectrograph manufactured by the Physical Institute of the Leningrad University. It has optical parts made of glass and a dispersion of from 50 A to 1 mm. in the violet region. Occasionally for the sake of approximate quantitative comparison fluorescence spectra of standard hyrocarbon solutions in benzene were photographed on the same plate. The arrangement of the bands in the spectrum of a benzpyrene solution in benzene at a concentration of 0.5 to 1γ in 1 cc. as obtained under our conditions of photographing is shown in Table I.

Table I: Fluorescence Spectrum of Benzpyrene Solution in Benzene at Concentration of 0.5 to 1γ in 1 cc.

I	II	III	IV	V
4,030-4,080 4,055	4,100-4,120 4,110	4,170-4,190 4,180	4,250-4,430 4,300	4,520-4,680 4,580

The width of the bands, particularly the position of their long-wave edge is somewhat dependent on both concentration and exposure. The short-wave edge of the bands is more definite. Band IV is the most intense, band I ranks second, then come bands V and II, while band III takes the last place. At the lowest concentrations only I and IV prove to be different (Fig. I).

The Distribution of Benzpyrene in the Body when Introduced into the Blood or Subcutaneously[1]

Hydrocarbon was introduced into the blood in a

[1] Investigations carried out by Assistant Physician I. Plindov.

fine aqueous suspension prepared by precipitation from acetone according to Boyland's method (4). By means of careful evaporation the suspension was reduced to the desired concentration. One of the series of experiments carried out on mice is further described.

Each of 20 mice was given 1 cc. of an aqueous suspension of 0.3 mgm. benzpyrene administered into the caudal vein. The mice were killed in pairs after different intervals. The blood of every pair was then collected, as well as their lungs, liver, kidneys, spleen, the adipose tissue from the peritoneal cavity (chiefly that surrounding the testes), and the small and large intestine together with its contents. 0.5 of each organ was used for preparing the extracts which were then reduced to an equal volume of 2 cc. and their fluorescence spectra photographed. The data obtained are shown in Table II (the plus signs designate the relative intensity of the bands in the spectrum).

TABLE II: THE DISTRIBUTION OF BENZPYRENE IN THE BLOOD AND ORGANS AFTER INJECTIONS

Time	Blood	Lung	Liver	Kidney	Spleen	Adipose tissue	Upper segment of the small intestine	Lower segment of the small intestine	The large intestine
15 minutes	+	+++	+++	+	+	+	++	+	+
30 "	+	+++	+++	+	+	++	++	+	+
1 hour	+	+++	+++	+	+	++	++	++	++
3 hours	—	++	—	—	—	+	—	+	+
6 "	—	—	—	—	—	+	—	—	+
12 "	—	—	—	—	—	+	—	—	+
24 "	—	—	—	—	—	+	—	—	+
48 "	—	—	—	—	—	—	—	—	—

Table II shows that within the first hour following the administration of benzpyrene its presence is revealed in varying quantities in all the organs and tissues examined. The lungs and the liver contain larger quantities of benzpyrene than any of the other organs (Fig. 2). The accumulation of benzpyrene in the lungs is accounted for by the fact that its larger particles may block the lung capillaries immediately after the suspension has been introduced into the vein, as was first noted by Peacock (18). On the other hand the concentration of hydrocarbon in the liver is evidently the result of the liver cells retaining the benzpyrene which has been dissolved in blood. Peacock's investigations (17) have made it generally known that the liver eliminates hydrocarbons together with bile. Later we shall return to the discussion of this phenomenon. As long as hydrocarbon circulates in the blood it is also found in the kidneys and the spleen in a concentration almost equal to that of the blood. An undoubted accumulation of benzpyrene takes place in the adipose tissue, which moreover retains it for a longer period of time (24 hours).[2]

As a result of benzpyrene being taken up by the viscera, eliminated with bile, as well as with urine (see below) and possibly partially destroyed, the concentration of hydrocarbon in blood is relatively low and in about 2 to 3 hours benzpyrene is found to disappear from the blood entirely.

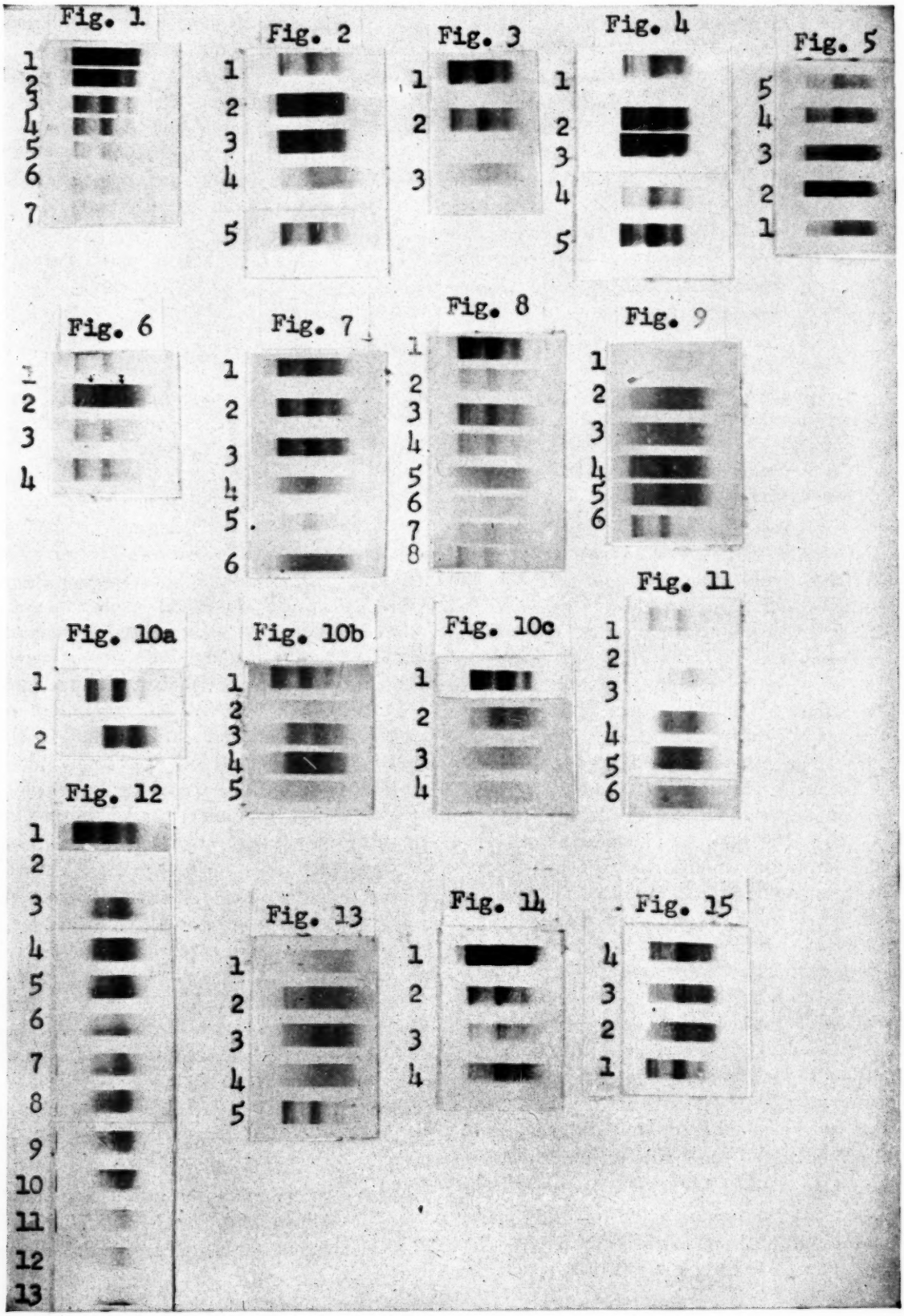
Similar results have been obtained on rats. The administration of 1 mgm. of benzpyrene into the blood was followed by its rapid disappearance from the latter (Fig. 3). Within the first hour large quantities of benzpyrene were found in the lungs and the liver,[3] smaller quantities in the kidneys and the spleen (Fig. 4), but in a short time (in 3 hours) it was no longer found in any of these organs. Hydrocarbon was found to be retained longest by adipose tissue (Fig. 5).

Several experiments carried out on rabbits revealed similar data. When 1 or 2 mgm. benzpyrene are given, the latter appears in the blood within the first 2 to 3 hours following its administration, and not only in whole blood, but in serum as well. Two experiments in which hydrocarbon was added to stabilized rabbit blood *in vitro* and the plasma and washed erythrocytes extracted separately have shown the coefficient of its distribution between the erythrocytes and the plasma to be practically equal to 1.

With respect to the intestine it can be seen from Table II (experiments on mice) that hydrocarbon is at first found to be present in all its segments

[2] The solution of benzpyrene in body fat was first described by Chalmers and Peacock (8).

[3] In one of the experiments in which 1 cc. of blood contained about 0.5γ benzpyrene the extract of 1 gm. of liver was found to contain a quantity of about 5γ.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(the intestines were extracted with their contents). Shortly after, however, it was found to disappear from the upper segment of the small intestine (simultaneously or immediately following its disappearance from the blood), and a little later from the lower segment. On the other hand benzpyrene could be determined in the large intestine for 24 hours (and in the case of rats for 48 hours).

The fluorescence spectra of the extracts drawn from the upper segment of the small intestine in mice proved to be different from those of the other organs: a wider and more intense band (from 4,150 to 4,210 A) was observed in place of the usually faint band III, while between bands IV and V a new diffuse band of about 4,420 to 4,500 A in width was found to appear. This latter band seemed to be less distinctly outlined and owing to its close proximity to band V of benzpyrene is almost fused with the latter (Fig. 6). It is evident that in this case an overlapping of two different substances—i.e. of benzpyrene and of its derivative—has taken place. The position of the latter's bands approximately coincides with that of the benzpyrene derivative which is eliminated with bile (see also below), and obviously enters the intestine together with it. The fluorescence spectrum of the

DESCRIPTION OF FIGURES 1 TO 15

FIG. 1.—Fluorescence spectra of benzpyrene solutions in benzene in decreasing concentration 1 = 2.5γ (in 1 cc.), 2 = 1.0γ, 3 = 0.5γ, 4 = 0.25γ, 5 = 0.1γ, 6 = 0.05γ, 7 = 0.025γ. Ten minute exposure.

FIG. 2.—Fluorescence spectra of benzene extracts from the viscera and tissues of mice in 1 hour following an intravenous injection of 0.3 mgm. benzpyrene. 1 = blood, 2 = lung, 3 = liver, 4 = kidneys, 5 = adipose tissue.

FIG. 3.—1 = benzene extract from blood in 30 minutes following intravenous injection of 1 mgm. of benzpyrene, 2 = in 1 hour, 3 = in 3 hours.

FIG. 4.—One hour after injection of 1 mgm. benzpyrene: 1 = blood, 2 = lung, 3 = liver, 4 = spleen, 5 = adipose tissue.

FIG. 5.—Benzpyrene spectrum in an extract from the adipose tissue of rats. From bottom to top: 1 = in 30 minutes, 2 = in 1 hour, 3 = in 3 hours, 4 = in 6 hours, 5 = in 12 hours after an intravenous injection of 1 mgm.

FIG. 6.—1 = benzpyrene solution in benzene (0.1γ in 1 cc.), 2 = extract from upper portion of small intestine, 3 = extract from lower portion, 4 = extract from large intestine—30 minutes after an intravenous injection of 0.3 mgm. benzpyrene in mouse.

FIG. 7.—In an hour after an intravenous injection of 1 mgm. benzpyrene to rat: 1 = wall of the upper portion of the small intestine, 2 = wall of the lower portion, 3 = wall of the large intestine, 4 = contents of the upper portion, 5 = contents of the lower portion, 6 = feces.

FIG. 8.—Standard benzpyrene solution in benzene (0.5γ in 1 cc.), 2 = wall of the upper portion, 3 = wall of the lower portion of the small intestine, 4 = wall of the large intestine, 5, 6 and 7 = contents of these portions of the intestine respectively, 8 = benzpyrene solution in benzene (0.1γ in 1 cc.) .

FIG. 9.—Twenty-four hours following subcutaneous injection of 1 mgm. of benzpyrene in mouse: 1 = blood, 2 = liver, 3 = lung, 4 = large intestine, 5=adipose tissue, 6 = standard benzpyrene solution in benzene (0.1γ in 1 cc.). Benzpyrene bands in extracts of the large intestine and of adipose tissue are visible.

FIG. 10a.—1 = control benzpyrene spectrum, 2 = spectrum of an extract of rabbit urine collected in 2 hours following an intravenous injection of 1 mgm. benzpyrene.

FIG. 10b.—Analogous experiment on another rabbit: 1 = control benzpyrene spectrum, 2 = rabbit urine previous to benzpyrene injection, 3 = in 1 hour following the injection, 4 = in 4 hours, 5 = in 6 hours.

FIG. 10c.—Experiment on mice (0.3 mgm. benzpyrene administered into the blood): 1 = control benzpyrene spectrum, 2 = urine in 3 hours' time after injection, 3 = urine in 12 hours, 4 = urine in 24 hours.

FIG. 11.—Fluorescence spectrum of whole bile in dog after an intravenous injection of 2 mgm. of benzpyrene: 1 = control benzpyrene spectrum (benzene solution), 2 = bile previous to benzpyrene injection, 3 = the same in 30 minutes' time after the injection, 4 = in 1 hour, 5 = in 2 hours, 6 = in 3 hours.

FIG. 12.—Experiments on dog No. 3. May 26, 1941. Phosphorus injected on May 22, 24 and 25, 1941. 1 = control benzpyrene spectrum, 2 = fluorescence spectrum of whole bile in 30 minutes after an intravenous injection of 2 mgm. of benzpyrene, 3 = the same in 1 hour, 4 = in 2 hours, and then successively in 2 1/3 hours, in 3 hours, in 3½ hours, in 5 hours, in 5½ hours, in 6 hours, in 6½ hours, in 7 hours and in 7½ hours.

FIG. 13.—Extract of cholesterol deposit in a control rat, 2 = extract of cholesterol deposit in 3 hours after an intravenous injection of 1 mgm. benzpyrene, 3 = in 9 hours, 4 = in 20 hours, 5 = standard solution of benzpyrene in benzene (0.1γ in 1 cc.).

FIG. 14.—Fluorescence spectra of extracts from the stomach of rats (together with the gastric contents) after the administration of 1 mgm. benzpyrene in food: 1 = in 3 hours, 2 = in 6 hours, 3 = in 12 hours, 4 = in 24 hours.

FIG. 15.—In 6 hours after the administration of 0.2 mgm. benzpyrene in food to a mouse: 1 = extract of the stomach, 2 and 3 = extracts of the upper and the lower portion of the small intestine respectively, 4 = extract of the large intestine.

benzpyrene derivative eliminated by the liver and known as BPX[4] was first described by Chalmers. It is characterized by two wide bands. According to Chalmers (5) BPX, in an alcohol extract of the bile of mice, has bands at 4,100 to 4,250 and (4,350 to 4,500).[5]

Benzene extracts obtained either from the lower segment of the small intestine or from the large intestine usually were found to contain unchanged benzpyrene. Only in two of the cases BPX was discovered in these parts of the intestine: in 30 minutes (in the lower part of the small intestine) and in 24 hours (the contents of the large intestine) following an injection. In the case of rats no derivative, similar to the BPX observed in mice, was ever found to be present in the intestinal contents.

The presence of fairly large quantities of unchanged benzpyrene in extracts drawn from the intestine leads to the supposition that hydrocarbon may be retained from the blood by the intestinal walls.

With the view of settling this problem additional experiments were carried out. The contents of the intestine and its wall washed of the contents were extracted separately. In parallel experiments made on rats the vascular system of the animals was previously washed with normal saline solution by means of a cannula inserted into the heart. The experiments have shown that the intestinal wall actually retained benzpyrene. In the case of rats, in particular, the quantity of benzpyrene present in the wall of both the small and the large intestine an hour after its administration proved to be fairly considerable, while the intestinal contents had mere traces of benzpyrene, if any (Fig. 7).

In addition, an experiment was carried out on a rat in which the lower part of the duodenum was previously ligated. After the suturing of the abdominal cavity the animal was given the usual dose (1 mgm.) of benzpyrene intravenously. In 3 hours' time hydrocarbon was found to be present in the wall of all the segments of the intestine (the vascular system had been thoroughly washed), while the intestinal contents had only barely noticeable traces (Fig. 8). (The latter phenomenon may be accounted for either by a partial peeling off of the mucosa in removing the intestinal contents or by a partial excretion of hydrocarbon by the intestinal glands into the lumen of the intestine). In addition the retention of benzpyrene by the intestinal wall is confirmed by an experiment the author carried out together with N. N. Blokhin on a dog in which vascular cannulae were inserted (after London's method) into the portal vein and into one of the hepatic veins. In this case arterial blood was found to have a fairly high concentration of hydrocarbon 1 hour and 40 minutes after an intravenous injection of 5 mgm. of benzpyrene, while the blood of the portal vein contained but traces of it.

Experiments on Subcutaneous Injections of Benzpyrene

The investigations carried out by my collaborators Feldman and Furman (10) in 1940 helped to prove that if injected subcutaneously in a solution of slowly absorbable oil (olive oil) in a dose of 1 mgm. benzpyrene can be revealed at the site of injection for as long as 3 months. If injected in a fine aqueous suspension hydrocarbon is more rapidly resorbed (injected in a dose of 1 mgm. it was found to disappear in our experiments in a month's time; injected in a dose of 0.5 mgm. it disappeared in 20 days' time).

A series of experiments was carried out on 14 mice which were given injections of 0.2 and 1 mgm. benzpyrene in an aqueous suspension. Benzene extracts of the blood contained no hydrocarbon. Apparently, being slowly absorbed from the subcutis into the blood, benzpyrene is so intensely withdrawn from it by the viscera that it fails to be determined in blood by the methods we have used. The liver, when examined within the first 48 hours following the injection, occasionally contained traces of benzpyrene. In this case the fluorescence of the gall bladder was observed. Within 24 hours after the injection traces of benzpyrene were also found in the lungs (possibly due to the retention of dissolved benzpyrene). Traces or even more noticeable quantities of benzpyrene were also determined in extracts of the large intestine. Beginning with 6 hours after the injection benzpyrene was invariably present in the adipose tissue of the peritoneal cavity (in one of the experiments its quantity amounted to 0.2γ to 0.5 gm. of adipose tissue) (Fig. 9).

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[4] Berenblum (*Cancer Research*, **3**:145. 1943) and his collaborators have found BPX to be 8-hydroxy-3,4-benzpyrene.

[5] In his subsequent reports describing experiments carried out mainly on rats Chalmers (6) states that in an alcohol solution of BPX extracted from the feces BPX has bands at (4,250 to 4,400) and (4,500 to 4,600) A.

The Elimination of Carcinogenic Hydrocarbons by the Liver[6]

The experiments were made on rabbits and consisted of inserting a glass cannula into the animals's common bile duct, and ligating the gall bladder duct. The bile was collected at definite intervals both before and after injecting carcinogenic substances into the blood.

Fourteen rabbits were given intravenous injections of benzpyrene in doses of 1 to 2 mgm. in an aqueous suspension. The first traces of the fluorescent substances were revealed in those portions of bile which had been collected in 10 to 15 minutes, or sometimes 20 minutes, after the injection of benzpyrene. To judge by the intensity of bile fluorescence the most active excretion of the substance begins in 30 to 40 minutes, rarely in an hour's time after the hydrocarbon injection was given, and lasts for about 30 minutes. After that the intensity of bile fluorescence gradually decreases, and in 2 to 2½ hours, less frequently in 3 to 3½ hours after the beginning of the experiment it ceases entirely.

The benzene extract was prepared as follows: the bile was dried by heating, then reduced to powder and extracted by a small quantity of benzene at room temperature. The fluorescence spectrum revealed 2 intensely manifest wide bands, one between 4,150 to 4,320 A, the other between 4,400 to 4,600 A (occasionally there was a third band of between 4,700 to 4,800 A). According to Chalmers' data in the spectrum of an alcohol solution of the BPX bands are located approximately 100 A further from the shortwave part (at 4,250 to 4,400 A and 4,500 to 4,650 A).

Experiments with methylcholanthrene were made on 4 rabbits. When an injection of 0.5 mgm. of methylcholanthrene was given the discharge of the fluorescent substance lasted for 2 hours, when 1 mgm. was injected it lasted 4 hours, while an injection of 2 mgm. caused the discharge to continue 4 to 5 hours. A curve computed so as to draw a comparison with benzpyrene has a more gradual rise and fall.

Bile fluorescence produced by injections of 1, 2, 5, 6-dibenzanthracene has proved to be much less intense and it was barely possible to determine the time when elimination ceased by means of visual examination. We were prevented from photographing the spectra of bile fluorescence in these latter experiments for reasons that have been previously mentioned.

[6] Investigations carried out by A. Friedman.

The Excretion of Benzpyrene by the Kidneys[7]

Six rabbits were given injections of 1 mgm. benzpyrene in an aqueous suspension into the auricular vein. Urine was collected either in metabolism cages or drawn by means of a catheter. Mice were injected with 0.3 mgm. hydrocarbon into the caudal vein. The urine of 5 animals was collected as follows: it was allowed to run through a wire netting in the bottom of the glass jar which retained the feces. A mere visual examination of the urine collected within the first 4 to 6 hours in the case of rabbits and 3 hours in the case of mice showed a violet-blue fluorescence. Benzene extracts revealed a typical fluorescence spectrum with two broad bands of between 4,150 and 4,300 A and 4,420 to 4,600 A (Fig. 10). In case of higher concentration there appeared a third barely noticeable band of between 4,700 to 4,800 A. Thus, benzpyrene was found to be discharged with urine in the form of a fluorescent derivative, whose spectrum closely resembled that of the substance discharged with bile.

Do Carcinogenic Hydrocarbons Penetrate through the Hematoencephalic Barrier?[8]

Among the problems concerning the distribution of carcinogenic substances in the body the possibility of their penetrating into the cerebrospinal fluid is of particular interest.

Accordingly experiments were made on rabbits, dogs and cats with the view to investigating the problem. The method used in the experiments consisted in drawing the cerebrospinal fluid by means of suboccipital punctures at different intervals following an injection of a benzpyrene suspension into the blood. The fluid was first tested visually with respect to its fluorescence, and then the fluorescence spectrum was photographed by means of a spectrograph. In some cases a benzene extract of the fluid was made, which was also examined spectrographically. Ten experiments were carried out on rabbits. The interval of 30 minutes of 1 or 2 injections with an interval of 30 minutes of 1 or 2 mgm. benzpyrene. The fluid was drawn at intervals of from 1 to 3 hours following the injection. Normal cerebrospinal fluid was found to produce a very faint bluish-grey fluorescence. After an injection of benzpyrene a visual test of the fluid revealed that its fluorescence in no way differed from that of the control fluid. A

[7] Investigations carried out by I. P. Plindov.

[8] Investigation carried out by the writer together with M. Zalesskaya.

spectrographic examination of the fluid or its extract which was made in the case of 3 rabbits, failed to reveal any benzpyrene bands. At the same time extracts of the arterial blood of two rabbits produced a spectrum of benzpyrene fluorescence.

Similar experiments with doses of from 2 to 4 mgm. benzpyrene were also carried out on 3 dogs. The fluid was drawn at intervals of from 1 to 24 hours following the injection of hydrocarbon. A spectrographic study of the fluid did not reveal any benzpyrene bands. When 2 mgm. benzpyrene were injected into a cat no hydrocarbon was discovered in its cerebrospinal fluid after an interval of 3 hours (the blood test was positive).

Thus, these preliminary data have shown that in the larger laboratory animals benzpyrene when injected into the blood, at least when it is given in small doses, is not detected in the cerebrospinal fluid.

In this connection it seemed interesting to study the question of benzpyrene solubility in the cerebrospinal fluid (according to our data 100 cc. of blood plasma dissolves up to 4 to 6 mgm. of benzpyrene). To study the problem we added to 2 cc. of the cerebrospinal fluid of a dog 0.02 mgm. benzpyrene in an aqueous suspension. After allowing the liquid to stand for 3 hours in a thermostat at 37° C. it was thoroughly centrifuged. A visual examination showed a typical blue-violet fluorescence and a spectrographic examination—the usual bands of dissolved benzpyrene (the suspension of undissolved benzpyrene particles produces a greenish-yellow fluorescence and a correspondingly peculiar spectrum). Consequently the solubility of benzpyrene in the cerebrospinal fluid is by no means very low.

Only after these experiments had been carried out we learned about a paper published by Peacock (18) in which he stated that part of the benzpyrene injected into the blood of mice was found to penetrate into the central nervous system. Bergoltz (3) also found a quantity of benzpyrene in the brain of mice in which an intravenous injection of 6 mgm. of hydrocarbon in ol. persicarum was made.

Do Carcinogenic Hydrocarbons Penetrate into the Milk?[9]

The question as to whether carcinogenic substances penetrate into the milk of a nursing mother is of great interest from the view point of its possible effect on the offspring, as well as in connection with the well-known fact that the action of tar and carcinogenic hydrocarbons increases the incidence of carcinomas of the mammary gland in mice (Soboleva [19], Larionow [12], Maisin and Coolen [15], and others).

We have carried out 2 experiments, one on a rabbit, the other on a dog. The female rabbit, which had had a miscarriage, was injected in the course of 3 days twice daily with 2 mgm. benzpyrene in fine aqueous solution into the aural vein. Milk was drawn daily from the mammary glands in quantities varying from 1 to 4 cc. at different intervals following the benzpyrene injection. The milk was extracted with benzene according to Berenblum and Kendal's method and the fluorescence spectrum of the extracts was then photographed. Only the spectrum of 1 of the 4 extracts obtained was found to exhibit a barely noticeable IV benzpyrene band, while the rest of the extracts had no benzpyrene bands at all.

The dog under experiment was left only one puppy to suckle. In the course of 3 days it was injected intraveonusly with 2 to 4 mgm. of benzpyrene in aqueous suspension daily. Milk was drawn every day (sometimes twice daily) for 4 successive days. During the course of benzpyrene injections the total number of milk tests taken was 6 (from 2 to 4 cc. each). Photographs of the fluorescence spectrum of benzene extracts from the milk did not exhibit any benzpyrene bands.

On the ground of these preliminary experiments we are apt to doubt that benzpyrene in an unchanged form was excreted with the milk of rabbits and dogs in any noticeable quantity.[10]

The Elimination of Benzpyrene by the Liver when it is Affected with Pathologic Processes[11]

A metal fistulous cannula was inserted under aseptic conditions into the gall bladder of dogs and the end of the cannula was made to protude through an incision of the skin. The common bile duct was ligated in the course of the operation. The incisions having healed, numerous experiments were carried out on the same dog.

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[9] Investigation carried out by the writer together with M. Zalesskaya.

[10] According to Peacock's (18) data, which became available to us only after we had made these experiments, when intravenous injections of benzpyrene were given to mice an excretion of hydrocarbon into milk was found to occur.

[11] Investigations carried out by A. Friedman.

Preliminary experiments carried out on 3 dogs showed that the animals were suitable objects for studying the elimination of carcinogenic hydrocarbons with bile for a period as long as several weeks and that the results they yielded were consistent. When 2 mgm. benzpyrene was injected into the blood, a typical blue-violet fluorescence appeared in portions of bile collected in 20 to 30 minutes following the injections, usually reached its maximum by the end of the first hour and mostly disappeared in 3½ hours (less frequently in 3 or 4 hours).[12]

Photographs of the fluorescence spectrum of whole bile revealed 2 diffuse bands peculiar to BPX. However, as compared to the benzene extracts of rabbit bile containing BPX (see above), these bands were situated nearer the short-wave part (approximately by 50 A), and besides, their margins (the short-wave margin of the first band in particular) were far more indistinct (Fig. 11).

Experiments on dogs showed besides that the portions of bile containing the fluorescent benzpyrene derivative rapidly turned green. Whereas normal bile preserved for a long time its olive-yellow color, bile discharging the benzpyrene derivative acquired within the first few hours a bright green, which was the more intense the greater the quantity of fluorescent substances it contained. Apparently, this acquired green color depended upon the oxidation of bilirubin into biliverdin which rapidly developed in the presence of the benzpyrene derivative excreted by the liver.

Experiments on the induction of pathological processes in the liver were carried out on 3 dogs. Hepatitis was brought about in 2 dogs by giving them injections of allylformate in the abdominal cavity. Control experiments carried out on the same dogs before they were injected with allylformate had shown that following an injection of 2 mgm. benzpyrene into the blood the elimination of its derivatives by the liver ceased in 3 to 3½ hours' time. Following the first injection of allylformate (0.15 cc. in 200 cc. of normal saline solution) no particular changes in the process of elimination were found to develop. After the second injection which was given 10 days after the first, both dogs soon exhibited a considerably lengthened period of eliminating the benzpyrene derivative with the bile. When 2 mgm. hydrocarbon was injected, the elimination lasted for 6 to 7 hours. The most intense elimination began half an hour later than in control experiments. This lengthening of the elimination period is apparently connected with a decrease in the rate of bile excretion.

Indeed, the total amount of bile discharged during the 5 hour duration of the experiment was reduced to almost half its previous quantity; for instance in dog No. 2 on the average from 20 cc. to 11 cc. Thus, the benzpyrene derivative was eliminated both previous and subsequent to the lesion of the liver by the same quantity of bile but over a longer period of time. Thus, in dog No. 1 benzpyrene was eliminated in control experiments in 14 cc. of bile (in the course of 3 hours), whereas after the second injection of allylformate it was eliminated in 15 cc. (in the course of 6 to 7 hours). No fall in the intensity of bile fluorescence was observed. Apparently, the liver, in spite of its being affected with a pathological process, excreted about the same quantity of the hydrocarbon derivative.

By the end of the experiment the first dog developed fairly marked ascites. It was killed on the 24th day. The second dog died in 10 days following the second injection of allylformate. The autopsy of the first dog showed its liver to be greatly reduced in volume. A microscopic study gave evidence of hepatitis and perihepatitis with prevailing phenomena of atrophy of the liver parenchyma. In the second dog a microscopic examination also revealed hepatitis with phenomena of initial cirrhosis.

In the third dog the lesion of the liver (fatty degeneration) was induced by means of phosphorus (three subcutaneous injections of 0.4 cc. 1 per cent ol. phosphorati). In this dog as well, the duration of benzpyrene elimination was increased from 3½ hours first to 7 and then to 12 hours. The beginning of intense elimination was delayed by 30 minutes. Contrary to the experiments with allylformate, poisoning with phosphorus did not reduce the quantity of bile excreted in a time unit. Consequently, the fluorescent substance was discharged in far greater quantities of bile as compared with controls (on the average it was 12 cc. previous to the injection of phosphorus and 20 to 30 cc. following it). The extent of bile fluorescence was fairly marked, as shown both by a visual test and by spectrographic examination (Fig. 12). The impression was that fatty degeneration of the liver caused a larger quantity of fluorescent substances to be eliminated with the bile than that eliminated by a healthy liver. This was possibly due to a larger quantity of benzpyrene having been dissolved in the fats and lipoids of the liver. The appearance of the fluorescence spectrum of whole bile in no way differed from that of controls (Fig. 12). The dog

[12] Similar data were obtained in the case of injections of 5 mgm. methylcholanthrene.

died in a fortnight following the last injection of phosphorus. Microscopic examination revealed moderate diffuse fatty degeneration of the hepatic cells, characterized by the fatty droplets being very small in size.

The possibility of the benzpyrene, contained in the blood, penetrating into the local lipoid accumulations was investigated in the following experiment. Four rats were injected subcutaneously with 10 mgm. cholesterol in a fine aqueous suspension. In 3 weeks the rats were injected intravenously with 1 mgm. benzpyrene. Then one by one the rats were killed in 3, 6, 9 and 20 hours after the latter injection and benzene extracts were drawn from their cholesterol granulomas. The first 3 extracts were found to contain about 0.2γ benzpyrene (Fig. 13). Identical experiments on mice yielded similar results. In one of our experiments on rabbits which were injected intravenously with benzpyrene, it was found to be contained in their suprarenal glands, apparently in the lipoids of the cortical substance.

Administration of Carcinogenic Hydrocarbons through the Gastrointestinal Tract[13]

Experiments were made on rats and on mice. In one of the experimental series hydrocarbon (3,4-benzpyrene or 20-methylcholanthrene) was dissolved in sunflower-seed oil, in the other series it was dissolved in milk. In the latter case an aqueous suspension of hydrocarbon was added to the milk from 12 to 20 hours previous to administration. Before the experiment the animals were kept without food for about 12 hours. After that they readily drank several cc. of milk containing the hydrocarbon or ate bits of toasted bread (1 gm.) soaked in several drops of an oil solution of hydrocarbon. The animals were killed at different intervals after the intake of food, and their gastrointestinal tracts were then examined first visually in a filtered ultraviolet beam and then their benzene extracts were subjected to spectrography.

An examination of the stomach of mice and rats fed with hydrocarbon solution besides revealing the fluorescence of the gastric contents, also showed within the first hours, an intense fluorescence of the forestomach mucosa even after it had been thoroughly washed of contents.[14] The mucosa of the glandular portion of the stomach produced no fluorescence. Evidently hydrocarbon was prevented from penetrating into this part of the gastric wall by the mucous secretion with which it is coated. The forestomach continued to be fluorescent even when the gastric contents had ceased to produce any fluorescence. In additional experiments the phenomenon was observed as late as 48 hours following the intake of food. The capacity of forestomach mucosa to absorb benzpyrene and retain it for a considerable length of time—a capacity lacking in the mucosa of the glandular stomach—should be attributed to the structure and chemical composition of its epithelial coat (stratified squamous epithelium). This peculiar property fully explains why any attempt to induce tumors of the stomach by adding carcinogenic hydrocarbons to food resulted only in bringing about tumors of the forestomach but never of the glandular portion of the stomach (13, 16, 20).

In experiments carried out on mice, fluorescence of the gall bladder was often observed within the first few hours, which seems to indicate that benzpyrene absorbed into the blood for the most part in the stomach was eliminated by the liver.

The results obtained by spectographic examinations of benzene extracts from different portions of the gastrointestinal tract (together with their contents) performed at different intervals following the oral administration of 0.5 mgm. benzpyrene in oil solution to rats are shown in Table III (the number of plus signs corresponds to the intensity of the bands in the spectrum).

Table III: Distribution of Benzpyrene in Segments of the Gastrointestinal Tract of Rats after Oral Administration

Time	Stomach	Upper segment of the small intestine	Lower segment of the small intestine	The large intestine
3 hours	++++	—	+	±
6 "	++	++	++	++
12 "	+	—	—	+++
24 "	+	—	—	+

Table III shows the gradual migration of unchanged hydrocarbon into the large intestine. The presence of small quantities of benzpyrene in extracts from the lower segments of the small intestine as early as 3 hours, in spite of its being absent in the upper segment, should be attributed to the fact that the benzpyrene which was absorbed by the blood and included in the general circulation was retained by the intestinal wall (see above). The lasting presence of hydrocarbon in an extract from the stomach is due to its having been retained by the forestomach wall (Fig. 14). The results ob-

[13] Investigation carried out by B. I. Vigdorovich.

[14] The phenomenon has been described in a paper published by our collaborators Feldman and Furman.

tained in analogous experiments on mice which were given 0.2 mgm. benzpyrene are shown in Table IV.

TABLE IV: DISTRIBUTION OF BENZPYRENE IN SEGMENTS OF THE GASTROINTESTINAL TRACT OF MICE AFTER ORAL ADMINISTRATION

Time	Stomach	Upper segment of the small intestine	Lower segment of the small intestine	The large intestine
3 hours	+++	++	++	+
6 "	++	+	+	++
12 "	++	—	+	++
24 "	++	—	—	+

The fluorescence spectrum invariably revealed bands of unchanged benzpyrene (Fig. 15).

Benzpyrene taken in milk produced on the whole similar results. The liver extract in these experiments produced the fluorescence spectrum of benzpyrene as early as 3 hours after food was given. Identical experiments were made with methylcholanthrene and yielded similar results. As early as 3 hours methylcholanthrene was found to be present in the extract from the large intestine, where it apparently was first carried by blood circulation. The greater part of the hydrocarbon, however, was only gradually transferred in the course of 24 hours into the lower portions of the intestine and was excreted with the feces. Part of the methylcholanthrene was retained by the wall of the forestomach. Extracts of the intestine, examined spectrographically, produced the fluorescence spectrum of unchanged-methylcholanthrene (it was also present in the feces).

SUMMARY AND CONCLUSIONS

The distribution of 3,4-benzpyrene within the animal body was studied on mice and rats which were given intravenous or subcutaneous injections of benzpyrene in a fine aqueous suspension. For this purpose benzene extracts were made from the blood, the liver, the lungs, the spleen, the kidneys, the intestine and the adipose tissue. The fluorescence spectra of the extracts were photographed by means of a spectrograph.

The spectrographic method was likewise used for studying the elimination of benzpyrene derivatives with bile in rabbits and dogs, and its excretion with urine in mice and rabbits. The elimination of benzpyrene derivatives by the liver was also studied in dogs in which pathologic lesions of the liver (hepatitis, fatty degeneration) had been experimentally induced.

The possibility of benzpyrene penetrating through the hematoencephalic barrier was studied by examining the cerebrospinal fluid drawn from rabbits and dogs. Experiments were also carried out with the view of determining whether or not benzpyrene penetrated into the milk of a dog or a rabbit suckling its young. Finally, the fate of carcinogenic hydrocarbons administered into the gastrointestinal tract was also studied. The investigations were started in 1940 and interrupted by the war in 1941.

The following conclusions may be drawn:

1. If 3,4 benzpyrene in aqueous suspension is injected intravenously its larger particles block the capillaries of the lungs. The smaller particles which circulate in the blood are dissolved by it in a fairly short time (later the particles retained in the lungs are also dissolved). Judging by preliminary data the hydrocarbon is more or less equally distributed between the erythrocytes and the plasma. In a short time, however, hydrocarbon disappears from the blood.

2. Benzpyrene is taken up from the blood by the liver, where it is found to be present (in an unchanged state) in a far higher concentration and from where it is eliminated together with bile into the intestine, as a fluorescent derivative having a peculiar fluorescence spectrum. 20-Methylcholanthrene and 1,2,5,6-dibenzanthracene are also eliminated by the liver.

3. Some of the benzpyrene is excreted by the kidneys together with urine, as a fluorescent derivative having the same fluorescence spectrum as the substance excreted with bile.

4. Part of the benzpyrene circulating in the blood is retained by the intestinal wall.

5. A considerable part of the benzpyrene is dissolved in the body fat and is retained by it for a considerable length of time, thus forming a kind of depot in the adipose cellular tissue.

6. When introduced subcutaneously benzpyrene is also found to be present in the body fat as well as in the liver, the intestine and the lungs (in small quantity).

7. If small quantities of benzpyrene are injected into the blood it is not found in the cerebrospinal fluid of rabbits and dogs.

8. To judge by preliminary data no noticeable quantity of benzpyrene in the form of fluorescent substances has even been observed to be excreted together with milk by rabbits or dogs.

9. In experimental hepatitis in dogs the benzpyrene injected into the blood is eliminated by the liver for a longer period of time because of the reduced rate of bile excretion.

10. In experimental fatty degeneration of the liver, the length of the elimination period is also increased, as well as the quantity of the benzpyrene derivatives which is excreted by the liver.

11. When benzpyrene or methylcholanthrene, administered in fat or oil solution, are added to food a considerable part of the hydrocarbons does not undergo any changes in the intestine and is excreted together with the feces in an unchanged state.

12. The forestomach mucosa in mice and rats is capable of absorbing hydrocarbons and of retaining them for a considerable length of time.

13. Part of the hydrocarbons is absorbed from the gastrointestinal tract (partly even in the stomach) into the blood and can be found, for instance, in the liver.

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Induction of Ovarian Tumors in Mice by X-Rays*

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The production of ovarian neoplasms in female mice by x-rays was described in 1936 (7, 10). The tumors arose about 7 to 10 months following irradiation with single or multiple doses of 200 r to 400 r. These tumors were of 3 histological types: (a) granulosa cell tumors, (b) luteomas, and (c) tubular adenomas. Whether the gland or duct-like structures of some of these adenomas are related to arrhenoblastomas has not been determined. Frequently, different parts of the same induced tumor had a different appearance.

Beginning about 5 months after irradiation hyperplastic changes were present in the ovaries of most x-rayed mice not bearing tumors. The borderline between neoplasia and hyperplasia was indistinct (3). Brambell and Parkes (17) have carefully described the hyperplastic ovarian changes that follow irradiation but they terminated their experiments about 6 months after irradiation, and were apparently unaware of the preneoplastic character of these alterations.

During the past decade little attention was given to the induction of ovarian tumors by x-rays and it was questioned whether the bulky masses of ovarian cells, which are formed after irradiation, were truly neoplastic. Traut and Butterworth (19) noted a great similarity between ovarian neoplasms of women and the experimentally induced tumors of mice, and traced the development of granulosa cell tumors of mice to granulosa cells that escaped destruction by x-rays. Geist, Gaines and Pollack (13), the only ones to repeat our experimental work, produced ovarian tumors in 22 of 38 mice by a single exposure to 200 r. They believe that the neoplastic granulosa cells arise from undifferentiated cells of the ovarian parenchyma.

Among the problems remaining to be solved were: (a) Is irradiation of the entire body necessary for the induction of ovarian tumors or would irradiation of ovaries alone be sufficient? Is the irradiation of the pituitary or some other organ with or without irradiation of the ovaries necessary? (b) What is the smallest dose of x-ray necessary for the induction of ovarian tumors and what is the relationship between the x-ray dose and the rate of induction of these growths? (c) Are these growths autonomous? (d) Do they secrete hormones?

Affirmative answers to the third and fourth questions have already appeared in preliminary publications (5, 9) in which it has been shown that granulosa cell tumors are actually neoplastic, occasionally metastasizing to distant organs, and proving readily transplantable to other hosts of the same inbred line of mice; and that animals carrying the growths often exhibit secondary changes indicative of hormone production (5, 11). The luteomas also proved transplantable and the secondary changes in luteoma-bearing hosts suggest progestin secretion (12).

The following previously unpublished experiments, undertaken in 1936 to 1938 with Dr. H. Traut to seek an answer to the first question, remained inconclusive. However, since they disclosed some technical difficultites and have guided subsequent work they are worthy of brief record.

Irradiation of the pituitary region with shielding of the ovarian region.—Five mice, approximately 3 months of age, received 800 r over the upper third of the body and head including the pituitary region. They died at 9 to 11 months following irradiation. The ovaries of these mice contained ova and exhibited none of the changes that precede the development of ovarian tumors in mice that have been exposed to x-rays. Another 4 mice that had been similarly irradiated at 2 months of age died at 14 to 17 months after irradiation. Their ovaries exhibited only the usual changes of senility.

These experiments strongly suggest that irradia-

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tion of the pituitary gland with shielding of the ovaries does not produce ovarian neoplasms.

Irradiation of the ovarian region with shielding of the pituitary region.—Twelve mice approximately 4 to 7 months of age were irradiated with 450 r over the lower half of the body, including the ovaries, while the head and upper half of the body were well shielded. These mice died from 5 to 13½ months after irradiation. Postmortem examinations showed the characteristic degenerative changes and atrophy that follow irradiation but no regenerative changes. Numerous mice of these two experimental series, which died earlier of intercurrent disease, have been omitted from this survey.

It remains uncertain whether regenerative changes leading to ovarian tumors were merely delayed, and if so whether this delay was due to the more advanced age of the mice at the time of irradiation or to the shielding of the pituitary.

It became evident that meticulous care of the animal is of prime importance in the study of the production of ovarian tumors by x-rays because of the long induction period of these tumors and the possible interference by intercurrent diseases. It also seemed advisable to use young mice for such studies and to obtain more information on the relation of the age of mice and the dose of x-rays to the rate of induction of ovarian tumors. The experiments here described were undertaken to answer the latter questions and to find out if, and to what extent, the application of methylcholanthrene might modify the carcinogenic action of x-rays.

MATERIALS AND METHODS

Groups of mice 4 to 6 weeks of age were irradiated with 87 r, 175 r, and 350 r respectively. Additional groups of similarly x-rayed mice were also "painted" with methylcholanthrene and a third group of control mice was similarly painted with methylcholanthrene but received no irradiation.

All mice were F1 hybrids of Rf females and Ak males and were thus genetically alike. This stock was used because it has a low incidence of neoplastic diseases, because it appears relatively resistent to the usual intercurrent infections and because of its longevity.

The factors of irradiation were as follows: 140 kv., 5 m. amp., 30 cm. target skin distance, with an inherent filtration of 1 mm. of aluminum.

Methylcholanthrene was applied as follows: An area of skin of about 1 × 1.5 cm. was gently "painted" with a 0.5 per cent solution of methylcholanthrene in benzol, making 50 strokes with a fine brush. The paintings were done twice weekly at 8 different sites, in rotation.

Most mice were kept until natural death; a few were killed *in extremis*. Six mice in this series are still living at over 31 months of age and all have large ovarian tumors proved by laparotomy.

The incidence of neoplasms other than those of the ovary in these mice is the subject of a future report. Stimulation of the leukemogenic action of methylcholanthrene by pre-irradiation has already been described (8).

RESULTS

Table I shows the frequency and time of occurrence of ovarian tumors in the different groups of

TABLE I: THE INCIDENCE OF OVARIAN TUMORS IN MICE EXPOSED TO X-RAYS AND "PAINTED" WITH METHYLCHOLANTHRENE

Treatment: X ray dose, γ	Methylcholanthrene, weeks	Age at death in months: <5		5-6		7-8		9-10		11-12		13-14		15-16		17-18		19-20		>21		Total	
		+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
87	–		4				1				1		2		2		1		9		31	43	8
87	5		3		2		6		2	5	2	3	6	5	5	6	3	2				18	32
175	–				1		1			1			3		4		9		8		24	48	3
175	1				2				2	2	1	1	4		6		5	1			1	19	6
175	3		1		9		1	2		1	3		2		1		1			1		7	16
175	5		2		12		1	8		4	3	2	1	1	1	6	3	3		1		18	30
175	7 and 9		5		23			9		1	3	3	2	1	1							5	43
350	–		2		2			7			1	6	3	2	1	4				3		17	32	16
350	5		17	1	23			9						1	2							2	51
—	1		1						1				2		5		5		8		1	—	23
—	3						2		4				6		5		3		1		1	—	24
—	5				5		9		2		10	1	7		10		3					1	46
—	7		1		6		1		9		3		3	1								1	23
—	9		2		7		8		5		4											—	26

experimental animals. The results are summarized in Table II.

TABLE II: COMBINED DATA ON THE INCIDENCE OF OVARIAN TUMORS IN X-RAYED AND METHYLCHOLANTHRENE-TREATED MICE

Age at death in months	X-rayed		Mice x-rayed and painted*		Painted*	
	+	−	+	−	+	−
<5	0	6	0	28	0	4
5-8	0	12	3	105	0	38
9-12	6	6	19	23	0	38
13-16	15	3	33	18	2	40
17-20	30	0	13	4	0	20
>21	72	0	1	0	0	2
Total living longer than 5 months	123	21	69	150	2	138
Total living longer than 18 months	92	0	2	1	0	11

* With methylcholanthrene.

The first ovarian tumor in mice x-rayed only was found 9 months after irradiation and at 17 months of age every mouse had an ovarian tumor irrespective of the dose of irradiation (87 r, 175 r, or 350 r). The percutaneous application of methylcholanthrene in both irradiated and normal mice brought about an earlier death of the mice mainly because it had induced leukemia, and cutaneous, breast and lung tumors.

It likewise remains doubtful whether or not methylcholanthrene alone is capable of producing ovarian tumors. Two ovarian tumors occurred among the 138 mice treated with methylcholanthrene only. The microscopic appearance of the ovarian tumors and ovaries of these mice were indistinguishable from those of x-rayed mice. Ovarian tumors are practically nonexistent in normal mice of this stock. Most methylcholanthrene-treated mice died at a relatively early age and their early death might have masked a slight ability of methylcholanthrene to induce ovarian tumors.

According to the tables, ovarian tumors were noted at an earlier age in mice that had received the combined treatment of x-rays and methylcholanthrene, than in mice that were x-rayed only. However, most mice that received 87 r or 175 r died after 19 months of age and no biopsies were taken on these mice at an earlier age. The induced ovarian tumors are known to grow very slowly. Therefore, the data summarized in the tables yield no information as to the actual time of onset of the ovarian tumors in the mice x-rayed.

On the genesis of ovarian tumors. Comparison of x-ray-induced granulosa cell tumors with the nodules resulting from grafts of normal ovarian tissue in spleens of gonadectomized mice.—Biskind and Biskind (1) discovered that implantation of ovaries into the spleens of adult gonadectomized female rats is followed several months later by the development of tumor-like masses of granulosa cells. The theory which led them to undertake this work is as follows: Estrogens are inactivated in the liver (15, 21). The estrogen level of the blood is the pace-maker for the discharge of gonadotropic hormones of the pituitary. Hence draining all estrogens into the liver where they are inactivated would result in an increased discharge of pituitary gonadotropic hormones with enhanced and seemingly uncontrolled growth of granulosa cells. Biskind and Biskind called attention to the similarity of the granulosa cell growths thus induced to those produced by x-rays but they did not prove the neoplastic character of their growths. Their work has been confirmed by Li and Gardner (16) and by ourselves (6).

In order to determine autonomy of the ovarian growths induced by the technic of Biskind and Biskind it seemed essential to graft these growths into the subcutaneous tissue or some other site not drained into the liver. Accordingly numerous first generation ovarian growths produced by this procedure in the spleens of mice were grafted into the spleens of gonadectomized and into the subcutaneous tissue of normal mice. No difficulty was encountered in making intrasplenic subpassages of these growths in gonadectomized mice but in only 2 instances did we succeed in establishing a progressively growing, subcutaneous neoplasm readily transplantable into normal mice (6).

This finding led us to accept with Li and Gardner (16) the theory of Biskind and Biskind and to assume that pituitary stimuli might play a role in the induction of ovarian neoplasms by x-rays. Several workers have shown (2, 14, 18) that following irradiation with about 140 r to 400 r there is a disturbance in the ovarian cycle suggestive of diminution of estrogenic output sometimes terminating in an anestrous state. Estrus reappears, however, either (a) because of the regenerative changes which follow atrophy of estrogen secreting cells of the ovary or (b) because of a compensatory mechanism.

In the experiments of Geist, Gaines and Escher (14) the destructive effects of 200 r were accompanied by temporary abolition of estrus during the period of 4 to 8 weeks after irradiation. Subsequently the cycles became irregular with marked variations in the duration of estrus and in the intervals between

estrus. From 6 months to 1 year after irradiation there was a decrease in vaginal stimulation. After 1 year estrus was absent but it reappeared in animals that developed biologically active granulosa or theca cell tumors.

It seems probable that the diminished output of estrogens in x-rayed mice stimulates the discharge of gonadotropins by the pituitary and that these act as growth stimulants of granulosa and lutein cells. The discharge of hormones by these stimulated ovarian cells into the general circulation would then depress the pituitary and thus true neoplasms would not develop until a new type of granulosa or lutein cell arises which is emancipated at least in part from the growth control of the pituitary gland. Most, if not all, x-ray induced ovarian tumors are transplantable in the subcutaneous tissue of normal mice (6). They are, therefore, autonomous and not mere masses of normal granulosa or lutein cells. Most growths induced by the technic of Biskind and Biskind are not transplantable in the subcutaneous tissue and are to be regarded as extreme examples of hyperplasia, which however can lead to neoplasia, as indicated by the observations just described.

X-rays can elicit neoplasms in tissues that are not known to be directly under the influence of the pituitary gland (e.g., skin) and it is possible that ovarian neoplasms can also arise in the absence of pituitary stimuli. On the other hand the pituitary gland might play an auxiliary role in the induction of these neoplasms, in that it discharges cyclic growth stimuli to the ovary and could thereby magnify the tissue derangement brought about in this organ by x-rays.

One type of ovarian growth is probably not directly related to the pituitary. These are the tubular adenomas, most of which appear to be distinct from arrhenoblastomas, that produce male sex hormones and whose mother cells may respond to pituitary stimuli (4). Tubular adenomas are also transplantable but grow much more slowly than granulosa cell tumors and luteomas and are usually outgrown by the latter (6).

In order to test the validity of these views it will be necessary to ascertain (a) whether x-rays induce ovarian tumors in hypophysectomized mice; (b) whether added pituitary gonadotropic hormones hasten their induction; (c) and whether the common tubular adenomas of x-rayed mice do secrete hormones and are dependent on the pituitary gland.

Susceptibility of mice of various ages and of various stocks to the induction of ovarian tumors.—Because of the established success in inducing ovarian tumors by irradiation with x-rays in mice of weaning age (4 to 5 weeks), it is desirable that in future studies mice of this age should be used as reference standards. The relative susceptibility of younger and older mice and of mouse embryos remains to be determined. Experiments now in progress (6) indicate that following total irradiation with 150 r at 1 to 3 days of age palpable ovarian tumors appear in many mice. One group of 9 mice so irradiated was explored by laparotomy at 14 months of age and ovarian tumors measuring 4 to 7 mm. across were found in 6. The unpublished experiment mentioned earlier in this paper has shown that regenerative changes are delayed when the irradiated mice are mature young adults. Although the pituitaries of mice of the latter series were shielded we believe that the lack of regenerative change is due to age. These fragmentary observations suggest that the probability of development of ovarian tumors following general irradiation is great when mice are exposed to x-rays at birth up to about 4 to 6 weeks of age.

In our original study (10) mice of 3 different stocks were used; the present work was done with hybrids of 2 of these stocks. Geist, Gaines and Pollack (13) induced ovarian neoplasms in an unrelated stock. Since there are no negative experiments on record it seems probable that mice of most, if not all, stocks are susceptible to the induction of ovarian neoplasms by x-rays.

Ovarian neoplasms in x-rayed women.—The rare occurrence of granulosa cell tumors in women and the lack of data suitable for statistical analysis make it uncertain whether x-rays will produce ovarian tumors in women. X-rays have been extensively used to sterilize women. These irradiations are local and there are no experimental data to indicate that ovarian irradiation alone is sufficient to produce granulosa cell tumors, although we believe this to be true. Furthermore, most women exposed to x-rays are middle-aged and the available experimental data cast doubt on the possibility of producing ovarian tumors under such conditions.

However, a large scale human "experiment" is now in progress in which girls and young women have been exposed over the entire body to comparable doses of gamma rays. The observations of Shields Warren (20) indicate that the late effects of atomic bomb explosions are similar to those of x-rays and it remains to be seen whether this type of radiating energy is capable of producing in human beings the neoplasms, including ovarian tumors, which can be elicited in mice by x-rays.

SUMMARY AND CONCLUSIONS

Following irradiation of 4 to 6 week old mice with 87 r, 175 r, or 350 r, ovarian tumors began to appear when the mice were about 11 months of age. The frequency of these neoplasms increased with time and almost every mouse that lived 17 months developed a unilateral or bilateral ovarian growth, irrespective of the dose of irradiation.

These ovarian growths are compared as to pathogenesis and autonomous character with the hyperplastic nodules that result from implantation of normal ovaries into the spleens of castrated mice. It is concluded that the latter are not autonomous neoplastic growths (although they sometimes give rise to true neoplasms); while the x-ray-induced ovarian growths, on the contrary, are readily transplantable autonomous growths.

The factors necessary for the induction of ovarian growths in mice and the bearing of the observations made on the general problems of carcinogenesis are discussed.

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Transplantable Luteoma in Mice and Associated Secondary Changes*

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From the standpoint of oncology lutein cells are of an unusual sort: they are not present at birth and appear when pituitary stimuli awaken the ovary to cyclic activity. Their mother cells are those surrounding the ova, and following completion of a cycle they gradually vanish. They are commonly regarded as granulosa or theca cells modified by hypophyseal hormones. This modification is profound, for with it goes the acquired ability to produce progestins, not elaborated by the mother cell. It has been doubted that they are capable of independent existence and of giving rise to tumors.

The experiments to be described indicate that the lutein cell can undergo a neoplastic change, and when this happens it becomes of a fixed type, and its relation to granulosa cells is no longer apparent. This neoplastic cell can retain the power of secreting hormones—seemingly discharged continuously and in large quantities—leading to secondary changes hitherto not definitely associated with these cells.

The very existence of luteoma is under controversy (6, 23 ,42, 47, 48). The number of cases of luteoma on record is small, and the authenticity of many of the cases reported is uncertain. Most workers suppose that luteomas are the irreversible end-phase of the luteinization of granulosa cell tumors and therefore not a distinct neoplasm (36). The masculinizing luteomas are generally believed to be adrenal cell rests.

The experimental production (18) and transplantation (17) of luteomas has led to a better understanding of this hitherto debatable tumor.

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Origin and Transplantation of Luteoma (Strain IX)

The luteoma from which strain IX originated was induced by irradiation with 175 r of a 5 week old female mouse, an Rf/Ak hybrid. During the week following irradiation, this mouse was also "painted" twice with a 1 per cent solution of methylcholanthrene in benzene (20). About one year after irradiation a laparotomy was performed and a yellow, soft tumor, found at the site of the left ovary, was removed with part of the uterine horn. This tumor measured 1.8 × 1.5 cm. in its two greatest diameters. The right ovary appeared normal; the left uterine horn was thickened, measuring 3 to 4 mm. in diameter. Two days later the mouse was found dead, and because of advanced postmortem decomposition it was discarded without removing tissues for extensive microscopic study. A small nodule present in the lung proved to be a papillary adenoma.

The ovarian tumor was cut into small fragments and injected into the subcutaneous tissue of normal and pre-irradiated mice.

Table I shows that the results of the transplantation experiments were successful in the original passage and that the takes varied from 0 to 100 per cent in later passages. The Table indicates that the luteoma is transplantable about equally to mice of both sexes. In the course of successive transplantation there was a conspicuous increase in the growth rate of the tumor cells as indicated by the decrease of both the incubation and tumor-bearing periods.

The cause of the failures of some attempted passages is obscure. It may have been due to poor selection of tumor material; e.g., inclusion of some necrotic parts in the inoculum, or lack of genetic uniformity of the recipient mice. The ovarian tumors were originally induced in Ak/Rf hybrids and

TABLE I: RESULTS OF IMPLANTATION OF LUTEOMA INTO RELATED MICE

No. of passage	Success of implantation ♂	Success of implantation ♀	Incubation period, days ♂	Incubation period, days ♀	Tumor-bearing period, days ♂	Tumor-bearing period, days ♀
I	4/4	5/5	83-350 (242)	116-167 (134)	K 154,183 D 117	K 33, 140
IIa	3/4	2/2	121-267 (198)	64-146 (104)	K 215-233 (224)	D 129,283
IIb	3/3	3/3	45	45	D 66-124 (88)	D 40-87 (68)
		#6/6		#45-58 (49)		#D 40-109 (69)
IIc	7/7	9/9	38-64 (53)	38-165 (71)	D 71-122 (85) K 47, 156	D 62-115 (84) K 112, 123
	*1/2	*2/2	*115	*81	*D 129	*D 53-79 (66)
IId	2/8	6/9	125,160 (143)	77-117 (109)		D 22-60 (39)
IIe	6/7	1/6	10-67 (33)	37	D 42-53 (48) K 73, 120	D 43
IIf	3/5	7/8	73-95 (84)	45-72 (56)	K 100, 146	D 68-110 (88) K 124
IIIa	3/6	1/7	14-29 (19)	56	K 97-118 (109)	D 56
IIIb		8/9		23		D 27-51 (39) K 39, 82
IIIc	#2/6		#60,75 (68)		K 19 D 57	
IIId	#0/2	#4/6		#35-69 (52)		#D 68-89 (80)
		4/4		58-69 (61)		D 82-90 (86)
IIIe	1/7	5/7	56	36-56 (44)	D 48 K 35-146 (93)	D 28-54 (41)
IVa	6/8	13/14	37-110 (59)	21-74 (48)	D 56	
IVb		1/6		46		K 58
V	7/7	0/4		39-82 (49)	K 24-25 (35) D 45-101 (73)	

All recipient mice were of same stock in which luteoma arose and were about 5 to 13 weeks old at time of injection. The denominator indicates total number of mice injected with tumor cells and the numerator, the number in which the grafts took. The incubation period is the interval in days between injection and appearance of tumor.

The table does not list 4 x-rayed mice injected during Passage I, all of which were grafted successfully; and 10 x-rayed mice of Passage IIa, 4 of which were successfully grafted.

A few figures on incubation and tumor-bearing periods were omitted because they were not available or not dependable.

= mice subjected to gonadectomy at about the time of injection.

* = these implantations were intra-splenic, all others subcutaneous.

K = Killed. D = died.

as recipients Ak/Rf hybrids were used. However, both the Ak and Rf inbred lines were broken down into sublines several years ago. In the present experiments no special consideration was given to these sublines and therefore the recipient animals were related but not of a single line.

The original transfer was also made into 4 female mice that had been irradiated with 350 r over the entire body 4 months prior to injection. The tumors in these mice were palpable 85 days after transplantation, a latent period considerably shorter than that in non-x-rayed young female siblings in which the tumor was palpable in 116 to 167 days (average of 134). In the second passage (IIa) a luteoma was also grafted into six 14 months old male mice that had been irradiated 9 days before with 350 r and into four 10 month old female mice irradiated with 350 r 8 months earlier. In the former group tumors appeared in two mice after 116 and 178 days respectively; and in the latter, in two mice after 16 and 33 days respectively. These periods are again shorter than the incubation periods in normal mice of the same passage, which averaged 198 days in males and 246 days in females.

The rationale of grafting tumor cells on x-rayed mice was as follows. Since the tumors arose in mice long after irradiation with x-rays it was thought that these tumor cells might proliferate in such mice but not in normal animals. Furthermore it is well known that x-rays enhance susceptibility to tumor grafts (35). However, since the tumors grew readily in normal mice of both sexes no further implantations were made in x-rayed mice.

Metastases were not observed in mice that received subcutaneous implantations of the tumor, but of 3 mice in which the tumors were grafted in the spleen 2 had metastases to the liver.

The interesting question arises: Why do these tumor cells metatasize from one site (and thus appear malignant) and not from another site (and thus appear benign)? The pattern of local circulation may account for the difference, but it seems more probable that the luteoma cells degenerate in the lung, which is reached by cells escaping from

subcutaneous grafts, and fail to pass the pulmonary barrier; while the liver, which is in the pathway of neoplastic cells escaping from the spleen, is a good soil for proliferation of luteoma cells.

The morphology of the splenic grafts is similar to that of the subcutaneous grafts, and the splenic grafts produce the same secondary changes. The livers of mice with luteoma grafts in the spleen are slightly more congested and there are small areas of necrosis in liver cells.

The grafted tumors grew slowly but progressively: regression was not observed after the transplants became palpable. The long incubation period, averaging in the primary passage 242 days in male and 134 days in female mice, is worthy of emphasis. The subcutaneous tumors usually reached 4 cm. and in a few cases 6 cm. in greatest diameter without interfering with the health of the animals.

The Morphology of Luteomas

The luteomas are slow-growing tumors, characterized in the gross by a faint yellowish hue, somewhat glossy appearance, and by a soft rubbery consistency. The deep yellow color of the induced luteoma was absent in transplanted luteomas.

The tumor cells (Figs 1, 2, 4, 6, 7) resemble very closely normal lutein cells (Fig. 3). They are polygonal with spherical nuclei and in contrast to all types of granulosa cells their cytoplasm is abundant and distinctly acidophilic. Cells with non-vacuolated, non-granular, and moderately eosinophilic cytoplasm are regarded as "healthy" (Figs. 6 and 7). They usually contain a much smaller quantity of sudanophilic lipoids than cells with more abundant and vacuolated cytoplasm which are believed to be degenerated (Fig. 8). The ectoplasm of the lutein cells is usually distinct, whereas the cytoplasmic borders of the granulosa cells are indistinct often causing the latter to appear as crowded masses of spherical, basophilic nuclei resembling those of large lymphocytes with little or no cytoplasm. The nuclei of the lutein cells are similar in size to those of granulosa cells but they are less basophilic. The deposition of large quantities of sudanophilic lipoids goes with further enlargement of the cell, assuming a pale-staining, vacuolated appearance in the usual hematoxylin-eosin preparation. There is a moderate variability in the size and shape of luteoma cells and their nuclei, but mitotic figures are rare. The only distinct stroma of the tumor appears to be a capillary network with vessels varying greatly in size in different parts of the tumor. The presence of thrombi in vessels with cavernous dilatation is common. Areas of hemosiderosis occasionally seen, indicate antecedent hemorrhage. Small focal areas of lymphoid infiltration occur (Fig. 7) but the bulk of the tumor is free from a cellular inflammatory reaction. Areas of necrosis are common in older tumors with occasional foci of calcification (Fig. 2). However, side by side with such calcified areas are well preserved masses of cells with no retrogressive change, or inflammatory reaction. Fig. 9 shows the margin of a necrotic area with clefts of empty spaces resembling those left by dissolved cholesterol crystals with no inflammatory reaction but with occasional giant cells, and adjacent typical luteoma cells. Reticulum is in general scant in luteomas and surrounds small or large groups of lutein cells. Rarely, there are cyst-like spaces in the tumor containing serum-like precipitate and a few red blood cells.

In the course of the passages the tumor became moderately anaplastic with occasional giant cells having hyperchromatic nuclei in some areas and with "dwarf" cells in others. The growth is invasive (Fig. 5) but metastases did not occur.

DESCRIPTION OF FIGURES 1 TO 5

All illustrations are from strain IX mice bearing luteoma, unless otherwise indicated. The tissues were fixed in Zenker-formol solution and stained with hematoxylin and eosin. The magnifications are approximate.

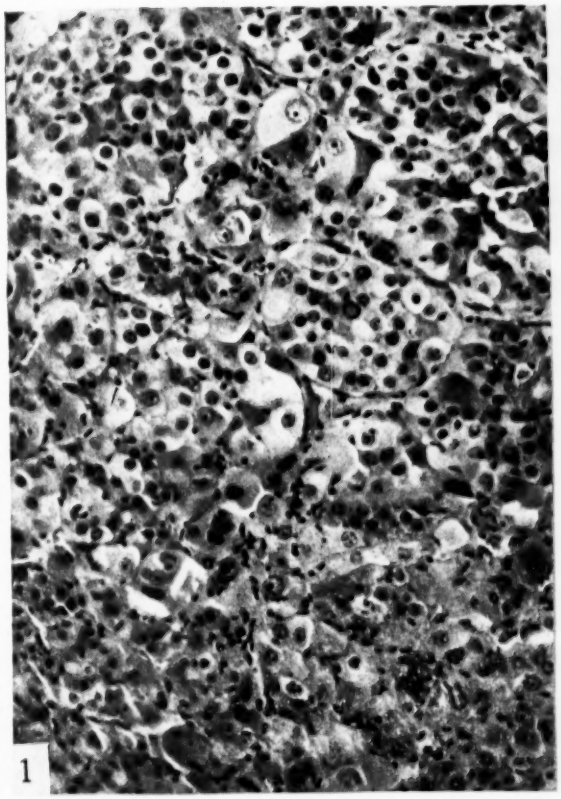
Fig. 1.—Luteoma of mouse Mb 1222 from which strain IX originates. Both nuclei and cytoplasm of cells vary greatly in size, but in general they resemble normal lutein cell. Mag. × 180.

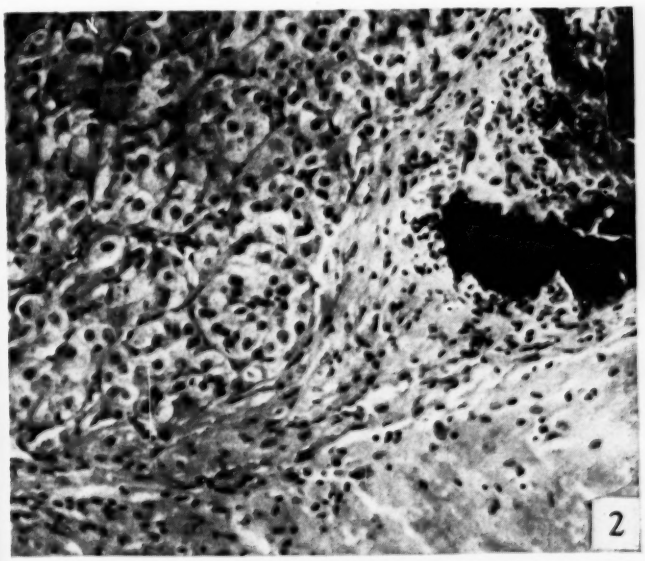
Fig. 2.—An area of degeneration from same tumor with deposits of dark staining material, presumably calcium, and slight fibrosis about well preserved lutein cells. Mag. × 180.

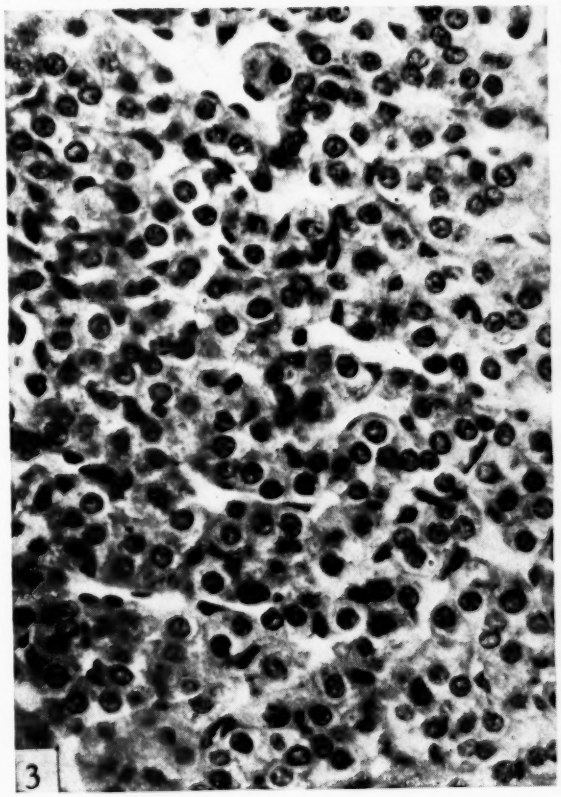
Fig. 3.—Corpus luteum of normal 4 month old mouse in diestrous. Note uniformity of size and shape of lutein cells. Mag. × 450.

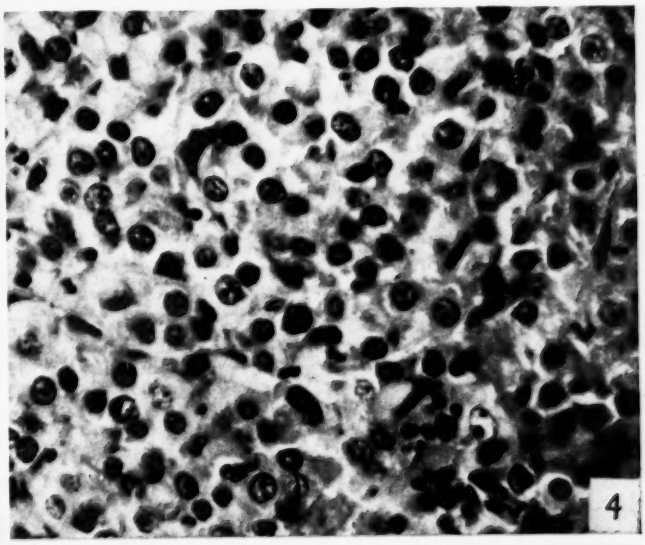
Fig. 4.—Transmitted luteoma (Passage 11c) of mouse Mc 787 ♀ grafted in spleen. Cells resemble closely normal lutein cells although there is a slight anaplasia. Mag. × 450.

Fig. 5.—Transmitted luteoma (Passage IIIc) of male mouse Mc 1054, infiltrating abdominal muscle. Grafted tumor measured about 3 cm. in greatest diameter when mouse was killed 4 months after implantation. Mag. × 180.









Changes in Mice Bearing the Transplanted Luteoma

The organs of tumor-bearing mice have been compared with those of normal mice; siblings or closely related mice weaned at the same time were used whenever possible.

The luteoma is associated with characteristic secondary changes in the sex organs, adrenal, thymus and submaxillary gland and frequently with obesity.

Obesity.—Mice bearing luteomas steadily gained weight in spite of the progressive growth of the tumor (Table II). The weight increase seems to have been due in part to a physiological growth which in this species does not cease after puberty. In normal control female mice of this stock the weight has increased from 21.5 gm. at 2 months to 32.7 gm. at 7 months. The gain in weight was somewhat less in mice that received implants but in which tumors did not develop. The weight of the tumor-bearing female mice had increased to an average of 40.1 gm. at 6 months: or to 12.1 gm. above that of uninjected controls and 16.5 gm. above those in which the tumor failed to grow. Similarly,

Table II: Weight Increment in Grams in Mice Bearing Luteoma

Mice		Age in months									
Females		2	3	4	5	5½	6	7	8	10	11
Uninjected	Number of mice	8	8	8	7		6				
	Extreme weights	17-23	19-27	20-29	21-29		24-30				
	Average weights	(21.5)	(24.4)	(26.2)	(26.9)		(28)				
Injected negatives	Number of mice	15	12	7	7		7				
	Extreme weights	16-28	21-28	21-27	20-28		22-29				
	Average weights	(22.2)	(23.9)	(23.3)	(23.6)		(23-6)				
Injected positives	Number of mice	0	3	8	6		6			3	3
	Extreme weights	–	24-27	27-31	32-36		38-43			50-61	52-62
	Average weights	–	(26)	(29.1)	(34.7)		(40.1)			(56-7)	(53.3
	Tumor size	–	+	+to++	+to ++++		++++			+++to ++++	+++to ++++
Weight increase of positives	Over controls		1.6	2.9	7.8		12.1				
	Over negatives		2.1	5.8	11.1		16.5				
Males											
Uninjected	Number of mice	14	14	14	13	13	10	3			
	Extreme weights	17-27	22-29	24-32	26-36	25-34	26-34	30-35			
	Average weights	(22.1)	(25.3)	(28.0)	(29.6)	(29.9)	(30.6)	(32.7)			
Injected negatives	Number of mice	16	10	6	6	6	3	1			2
	Extreme weights	19.28	23-32	26-30	25-32	25-39	31.33	–			35-41
	Average weights	(23.3)	(27.0)	(27.0)	(28.0)	(29.2)	(32.3)	(31)			(38)
Injected positives	Number of mice	2	8	12	11	11	4	2		3	6
	Extreme weights	25-26	25-31	27-37	32-39	32-41	35-39	34-38		42-44	40-48
	Average weights	(25.5)	(27.3)	(31.6)	(34.2)	(36.4)	(36.7)	(36)		(43)	(44.1)
	Tumor size	+	+to++	+to ++++	+to ++++	++to ++++	++to ++++	++to ++++		+to ++++	+to ++++
Weight increase of positives	Over controls	3.4	2.0	3.6	4.6	6.5	6.1	3.3			
	Over negatives	2.2	0.3	4.6	6.2	7.2	4.4	5.0			6.1

The values are pooled from Passages I, II, IIe, IIf and IIIa. + = Tumor up to 1 cm. in greatest diameter; ++ = tumor from 1 to 2 cm. in greatest diameter; +++ = tumors from to 3 cm. in greatest diameter; ++++ = tumor over 3 cm. in greatest diameter.

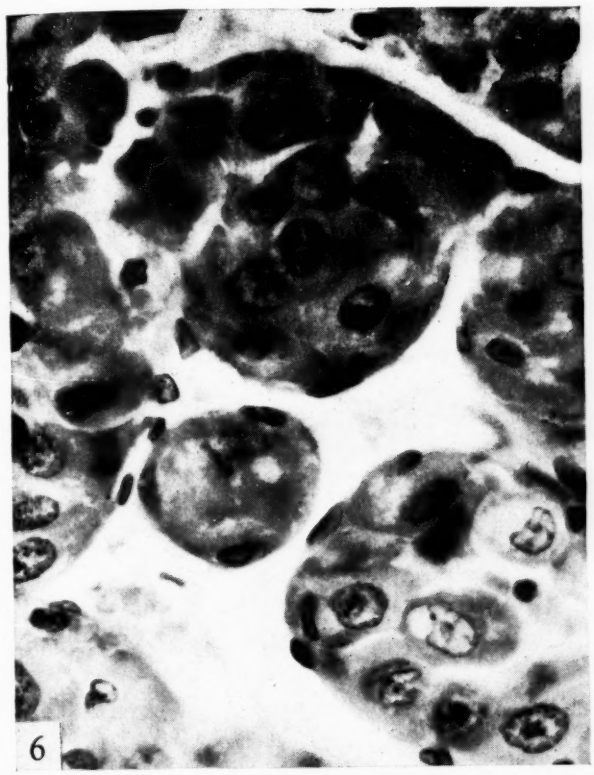
DESCRIPTION OF FIGURES 6 TO 9

Fig. 6.—High magnification of large transplanted luteoma (Passage IId) of mouse Mc 2015 ♀ 1 month old when implanted, 15 months old when killed. Mag. × 900.

Fig. 7—Transmitted luteoma (Passage IIe) of mouse Mc 806 ♂ showing mitotic figures and focal accumulations of lymphoid cells in tumor. Mag. × 450.

Fig. 8.—Another field of luteoma of mouse Mc 2015 ♀ with lutein cells that are vacuolated or have clear cytoplasm. Mag. × 450.

Fig. 9.—Transmitted luteoma (Passage I) of mouse Mc 421 ♂ showing necrotic area with clear spaces presumably those of dissolved cholesterol crystals. There is no inflammatory reaction and adjacent tumor cells appear well preserved. Mag. × 180.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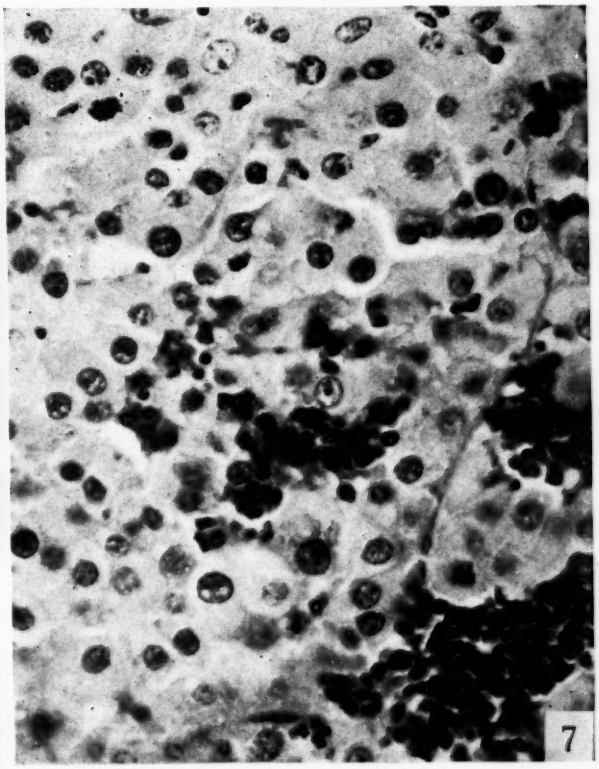
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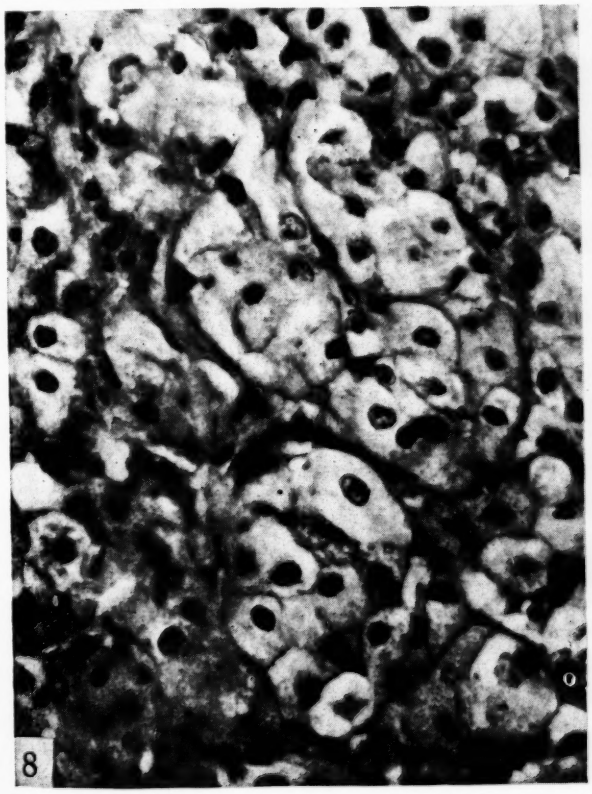
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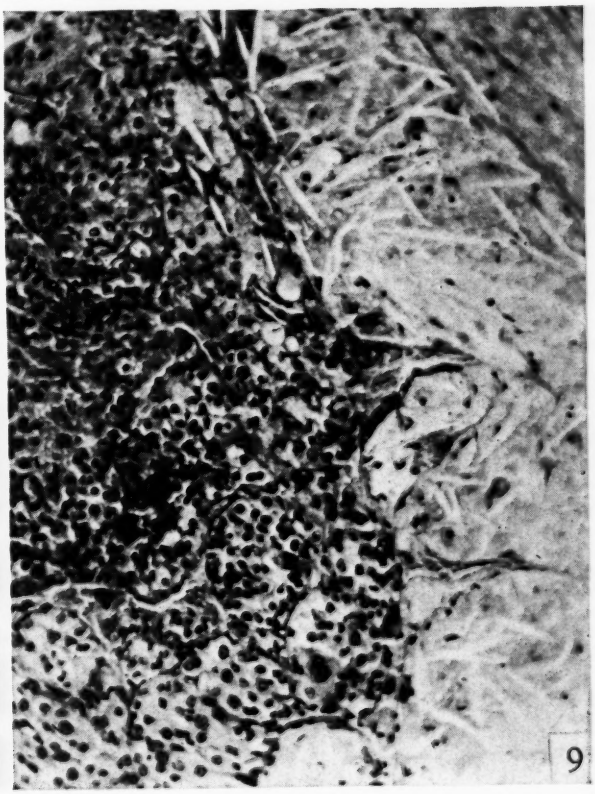
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tumor-bearing male mice gained about 6 gm. more than the normal controls. The weight increases in male mice may be due solely to the bulk of the tumor, while the weight increment in some females is more than the estimated sum of normal body weight and tumor weight. Unfortunately only a few tumors were weighed. The estimated weight of large tumors is 6 to 12 gm.

In contrast to mice receiving tumor grafts of different sorts, observed by one of us in the course of years of extensive studies of transplantable neoplasms, the luteoma-bearing mice were unusually obese. Terminally the weights tended to drop, but luteoma never produced cachexia.

Whether or not luteoma produces obesity remains to be investigated with other strains, since strain IX has increased in virulence in the course of successive passages and the associated obesity became less pronounced. In the original transfer the tumor growth was slow, and female mice reached a weight of 50 to 62 gm. at 10 months. No normal controls were available at that time. Subsequently the tumor grew faster and the weight increases were less obvious. Obesity was absent with progressively growing tumors. Fat was excessive not only in the subcutaneous tissue, but also in the internal fat depots. The fat-body about the kidney was so massive that in it the atrophic adrenal could be identified only with difficulty. Fat was excessive also in the thoracic cavity substernally and occupied the site of the atrophic thymus. The atrophic ovaries also were embedded in much fat.

The *vaginal epithelium* of tumor-bearing, noncastrate mice consists of 2 to 3 layers of cells. The lining cells are tall cuboidal or columnar with a basal nucleus and a pale pink staining bulky cytoplasm. The constant absence of estrogenic squamous metaplasia deserves emphasis.

The *clitoris* appeared larger in some tumor-bearing than in normal mice.

Vaginal smears of 3 ovariectomized and 3 normal females bearing luteomas, taken on 5 consecutive days, prepared and stained according to Papanicolaou's technic, indicated an anestrous state.

The *uterus* is enlarged, usually elongated, and measures about 3 mm. in diameter. Both musculature and epithelium appear hyperplastic, while the submucosa is scanty. The proliferating epithelial cells form papillary folds (Figs. 10, 11) and occasional downward projections into the muscularis (Fig. 12). The lining cells are predominantly tall columnar. The nuclei are crowded, but mitoses are rare. Many glands, notably those of the submucosa and muscularis are lined with cuboidal cells; some are cystically dilated, either devoid of or containing eosinophilic secretion. Isolated glands deep down in the muscularis are more common than in the uteri of normal mice. Occasionally they penetrate deeply and are seen beneath the serous coat (Fig. 12).

The uterus of the normal mouse differs in appearance, depending on the cyclic hormonal stimulation, as also indicated by the state of the vaginal epithelium. In general, the glands are small, and the muscularis appears thinner and is seldom invaded by glands. The stromal cells of the submucosa on the other hand are more numerous; their nuclei are round or slightly oval.

That the uterus is in a stimulated state in mice bearing luteoma is clearly indicated in tumor-bearing, spayed female mice in which the uterus is noticeably thicker than in non-tumor-bearing ovariectomized animals. The increase in size appears to be caused by hyperplasia of both muscularis and endometrium.

It deserves emphasis that the most characteristic effect of progesterone activity, namely, swelling and crowding of subepithelial stromal cells (28) is absent in luteoma-bearing mice; on the contrary, the submucosa is scant and the epithelium is hyperplastic. This discrepancy requires further study.

The *ovaries* of luteoma-bearing mice are invariably atrophic (Figs. 16, 17). Ova are present (Fig. 18) and they are surrounded by the usual cells of the follicles some of which contain liquor. Lutein cells,

DESCRIPTION OF FIGURES 10 TO 14

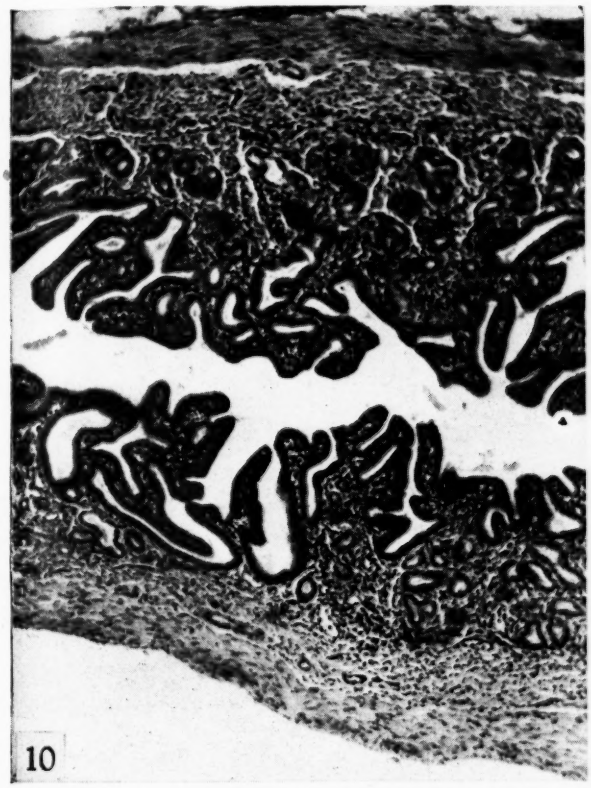
FIG. 10.—Uterus of mouse Mc 2022 bearing transmitted luteoma (Passage IId). Mouse was 1 month old when implanted and 4 months old when killed. There is hyperplasia of epithelium with hillocky surface. Mag. × 90.

FIG. 11.—Higher magnification of Fig. 10. Mag. × 180.

FIG. 12.—Downgrowth of endometrium of 6 months old mouse Mc 995 bearing transmitted luteoma (Passage IIf; grafted at 6 weeks of age), penetrating muscularis and reaching the serosa Mag. × 90.

FIG. 13.—Transplanted luteoma of strain III. Mouse Mb 1923 ♂ 10 weeks after implantation (Passage IIIa). Mag. × 450.

FIG. 14.—Another field of transplanted luteoma of strain III. Mouse Mb 1979 ♀ (Passage Va; 10 weeks after implantation). Thick fibrous connective tissue surrounds well preserved lutein cells. Mag. × 180.



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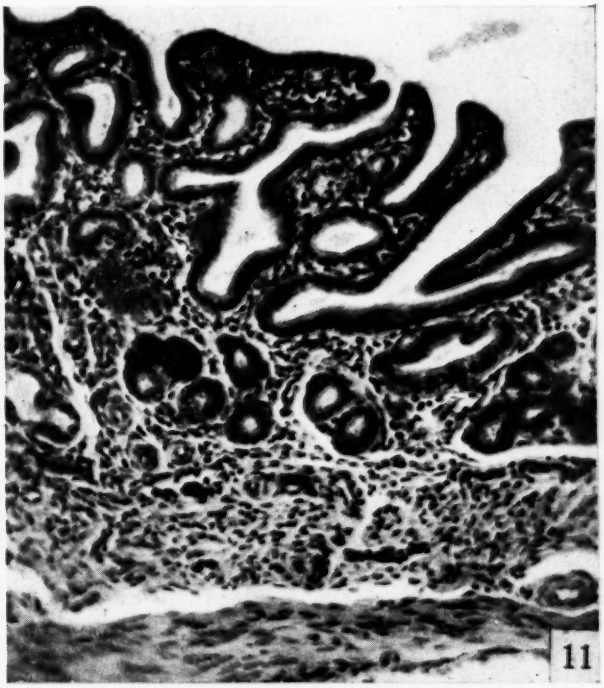

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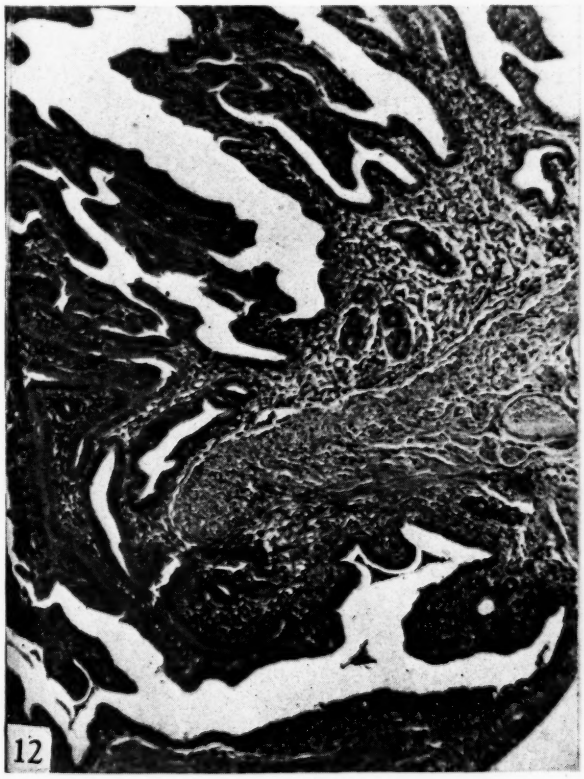

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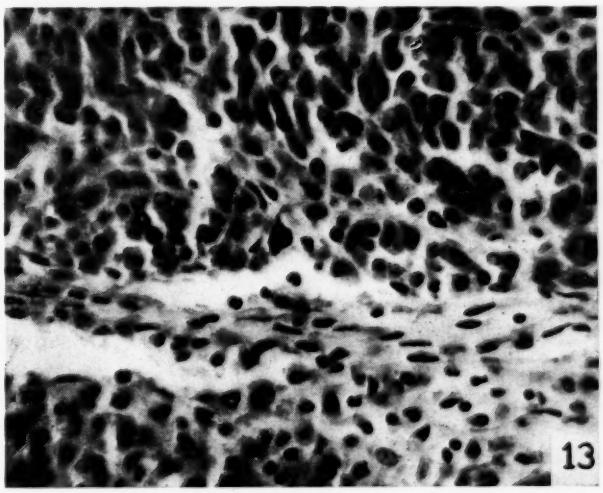

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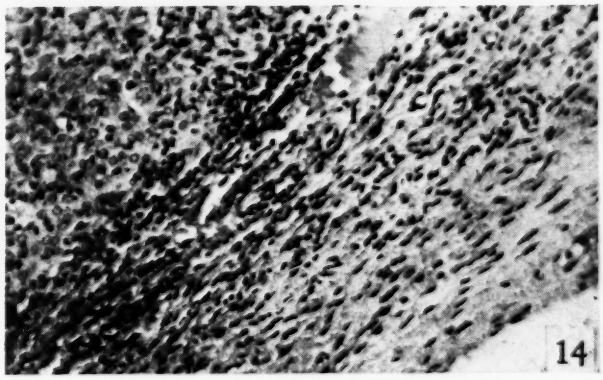

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however, are invariably absent. Many ova are degenerated, and the stroma appears scant or normal in amount (Fig. 16).

In the control animals corpora lutea are conspicuous (Fig. 15) occupying almost as much space as all the other ovarian elements combined. The cells of the corpora lutea (Fig. 3) in contrast to those of the luteoma exhibit a striking uniformity in size and shape and in their staining properties.

The *mammary glands* of tumor-bearing mice are as a rule fatty and atrophic. Some ducts are dilated with pink secretion, but acini are few or are absent. In an occasional mouse there was hyperplasia of ducts distended with pink-staining secretion.

In the *male sex organs* of luteoma-bearing mice no conspicuous changes were noted. Spermatogenesis was not affected, and the seminal vesicles and the prostate appeared normal.

Further evidence of secretion with androgenic effect. —The fact that progesterone is androgenic (24) prompted us to compare castrate, luteoma-bearing mice with control mice. Five pairs of tumor-bearing and control castrated mice were examined 1 to 5 weeks after castration. There was marked atrophy of the seminal vesicles in all controls and in none of the mice with luteomas.

In one experiment the tumor was grafted on male mice castrated 3 months previously. In both animals in which the luteoma grafts were successful, the seminal vesicles were greatly enlarged. Siblings castrated at the same time but not bearing the tumor were killed at the death of the tumor-bearing animals. The seminal vesicles of these mice were profoundly atrophic.

Characteristic adrenal cortical atrophy.—Atrophy of the adrenal cortex is a regular finding with luteomas in both sexes. The change was absent in only a few mice. It is associated with disappearance of the zona reticularis and of the regular arrangement of the fasciculate column with collapse or loss of cells of its medullary portion, and persistence of the glomerular zone (Figs. 19 to 22). Nuclei of cells in the atrophic portion of the gland may appear intact, but their cytoplasm is scanty. The medulla often appears large but is otherwise normal (Fig. 19). In a few mice there is a slight or moderate congestion of the deeper zone of the cortex and of adjacent medulla. Occasionally large, foamy "brown" cells are present in the midzonal region.

When the changes are advanced, the cortex is thin. All cells beyond the glomerular zone lose most of their cytoplasm, become flattened and crowded around the medulla (Figs. 21, 22). There is usually a sharp border between the chromaffin cells of the medulla and the cortical cells. Figs. 23 and 24 show the characteristic appearance of the normal adrenal cortex in mice of comparable age.

The *submaxillary glands* of female mice bearing luteomas have a characteristic appearance. Cells of the secretory tubules, like those of normal males, are distended with granular eosinophilic secretion and the nuclei are basal, while cells of acini are smaller. In normal females there is a predominance of acinar cells while the secretory tubules are in the minority and their nuclei are columnar and are not basal. The ducts are not distended with secretion.

Kidney. No definite masculinization of the kidney occurred in any of the luteoma-bearing female mice except one. In this mouse, bearing the largest tumor thus far observed, the glomeruli were predominantly of the male type. Congestion of glomeruli was common, and the lumina of the convoluted tubules frequently contained much pink-staining precipitate.

Liver. In the liver, changes in the parenchyma are insignificant. There is only slight or no congestion. In only one animal was a moderate congestion noted as seen with hypervolemia secondary to granulosa cell tumors (21). A moderate leukemoid reaction by myelocytes, erythroblasts and megakaryocytes is common.

Secondary changes in pre-irradiated mice bearing transplanted luteomas,—In animals pre-irradiated with approximately 350 r before grafting the luteomas, the changes are essentially the same as in normal animals.

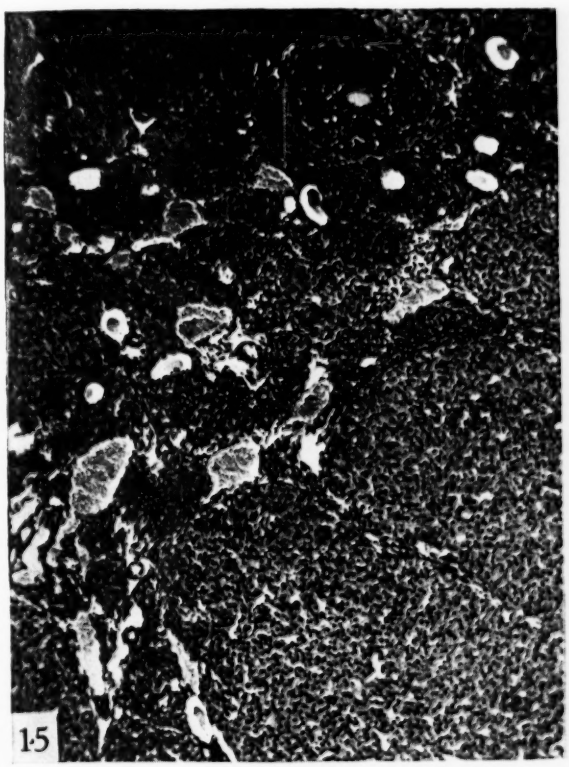
DESCRIPTION OF FIGURES 15 TO 18

FIG. 15.—Ovary of normal mouse, 4 months old, in diestrous, with characteristic follicles and several lutein bodies in lower corner of field Mag. × 90.

FIG. 16.—Ovary of mouse Mc 999, 7 months of age, bearing large transplanted lueoma (Passage IIf). Mouse was 6 weeks of age at implantation. Note well preserved follicles at periphery of field and degenerating ova and seemingly atrophic stroma in central part. Corpora lutea are absence. Mag. × 90.

FIG. 17.—Ovary and fallopian tube of mouse Mc 2022 bearing transplanted luteoma (Passage IId). Changes are similar to those shown in Fig. 16 (see Figs. 10 and II of same mouse). Mag. × 90.

FIG. 18.—Higher magnification of ovary shown in Fig. 17. Note normal appearing follicles and ova. Mag. × 180.



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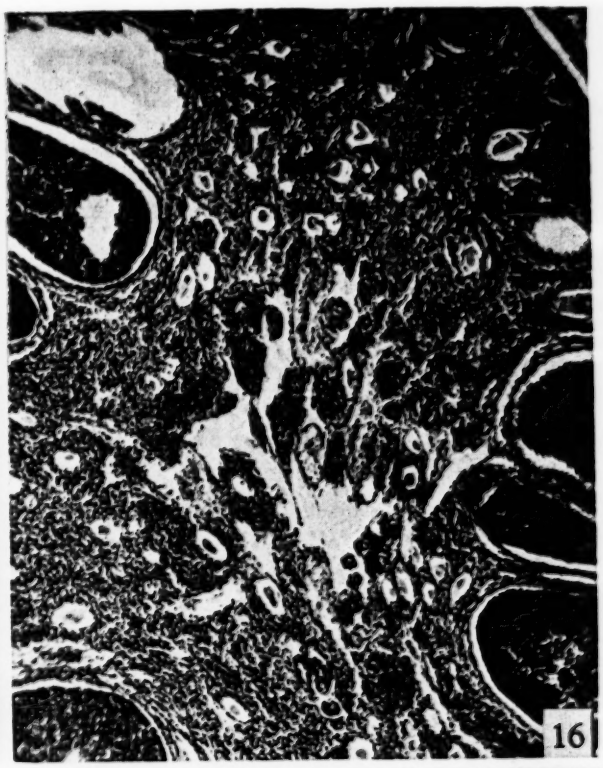

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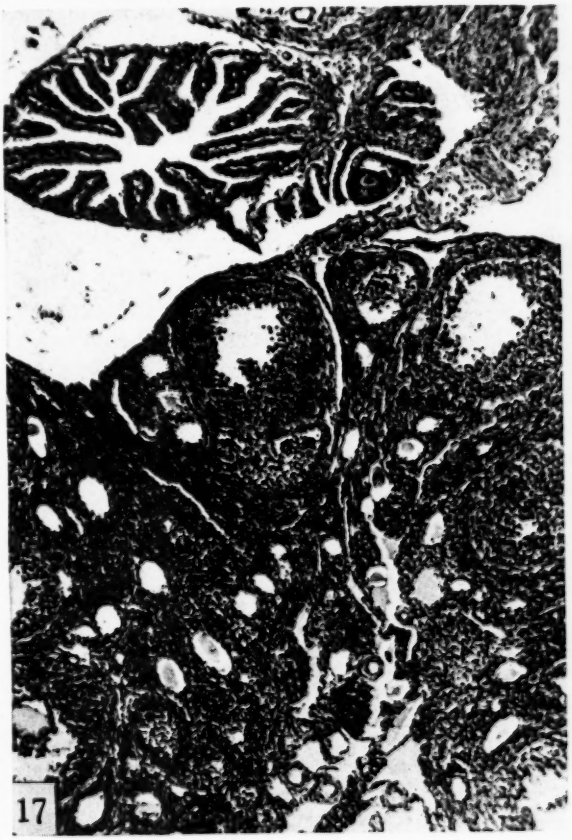

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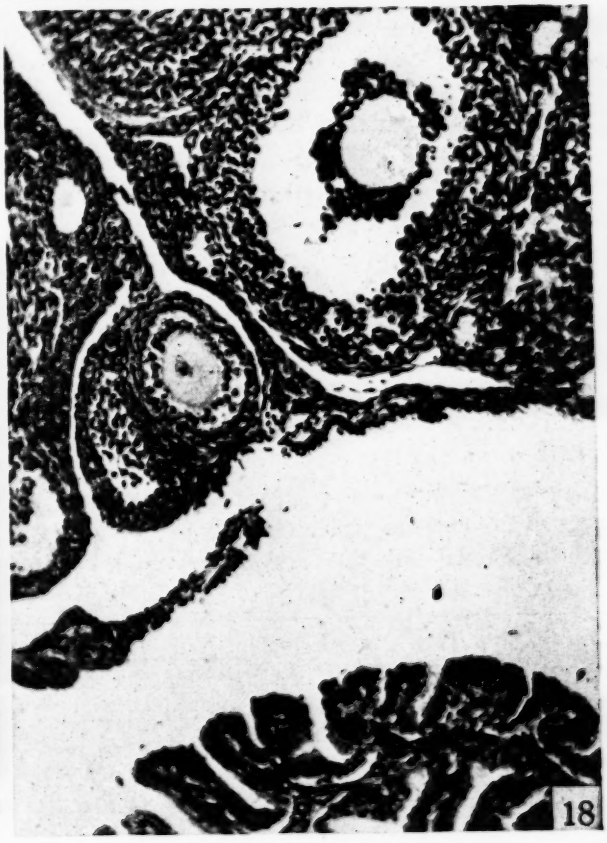

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DISCUSSION

Neoplasms of lutein cells.—The acquisition of neoplastic properties by lutein cells is of unusual interest, because this cell does not exist before puberty and its normal life is linked with the ovarian cycle. The neoplastic change endows it with heightened growth vigor and frees it from those hormonal forces that normally terminate its life. In this respect luteomas are tumors analogous to chorionepitheliomas. Both are composed of cells brought into existence in the course of the sex cycle and have a limited life span, but when they become a neoplasm they perpetuate their kind indefinitely.

The frequent occurrence of luteoma in mice that had been exposed to x-rays has already been described (18). Most x-ray-induced ovarian growths contain tumor-like masses of both lutein and granulosa cells. Both types proved transplantable and by successive passages of selected areas pure growths of either of these cell types can be obtained. Strain IX here described originated from a lutein cell mass and in the course of 16 passages it never yielded granulosa cells. The first successfully transplanted growths induced by x-ray were of the granulosa cell type (strain I, 19). From the third successfully transplanted ovarian tumor (strain III), which was of a mixed type, two pure lines of transplantable growths were secured; a transplantable granulosa tumor that produced hormones and a luteoma (illustrated in Figs. 13 and 14).

Existence of luteoma in women.—Luteomas in women are rare; they are poorly understood and many doubt their very existence.

According to Hoffman (27) only 12 cases of human luteoma have thus far been recorded. They may be benign or malignant. The youngest patient was 15 and the oldest 65 years of age. The tumor is bright canary yellow in color and the cells resemble those of a mature corpus luteum.

In order to diagnose a human luteoma it is necessary to exclude metastatic hypernephroma, growth of adrenal cell rests, and the possibility that the lutein cells are merely luteinized granulosa cells (23). The cytoplasm of cells of hypernephroma is clear, due to glycogen and to a lesser extent to fat, while that of lutein cells is filled with lipoids. According to Barzilai (6) lutein cells also contain glycogen finely dispersed in the cytoplasm. The resemblance to Krukenberg tumor cells is superficial. In the latter, clarity of cytoplasm is brought about by mucin, which is readily stainable with mucicarmine. The mucin apears to compress the nucleus to the periphery of the cell, whereas the vesicular nucleus of the luteoma is centrally placed, even when sudanophilic lipoids accumulate in the cytoplasm of the cells in large quantities.

Several cases of so-called human luteomas were associated with masculinization, hypertrichosis, voice changes, and amenorrhea (27, 44) and these tumors are believed to be derivatives of adrenal cell rests. Fig. D of Hoffman (27) showing the microscopic appearance of such a neoplasm, is indistinguishable from the luteoma described above (see also Fig. 3 of Furth and Butterworth, 18).

The morphological difference between lutein cells and lipoid-laden cells of the adrenal cortex of mice is slight; the lutein cells are somewhat larger and their cytoplasm is more bulky and eosinophilic.

Fekete and Little (16) noted the similarity between the prominent cells in tumors of the adrenal cortex produced in mice by castration (49), called Type A cells, and those of ovarian neoplasms. Their Figs. 1 to 4 bear a striking resemblance to granulosa cells of tumors induced by x-rays. Type B cells of the adrenal tumors (49) resemble both lipoid-laden cells of the adrenal cortex and lutein cells. The characterization of these cells requires identification of the hormones they secrete. These tumors are associated with both masculizing and feminizing hormones and it is possible that the Type A cell which resembles granulosa cells is the predominant source of the feminizing hormone and Type B cell of the masculizing hormone. It will still remain to be determined whether the latter is a progestin or a testoid substance. The isolation of transplant-

DESCRIPTION OF FIGURES 19 TO 24

FIG. 19.—Atrophy of adrenal cortex with intact medulla of mouse Mc 2015 ♀ bearing transplanted luteoma (Passage IId) (see Fig. 6 from same mouse). Mag. × 90.

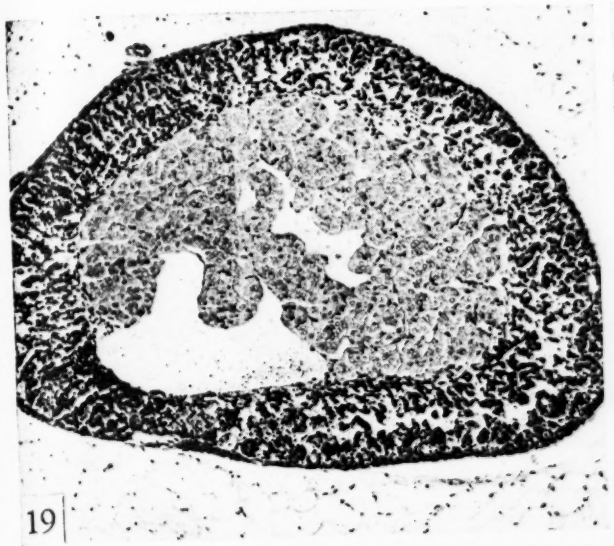
FIG. 20.—Higher magnification of Fig. 19. Mag. × 180.

FIG. 21.—Still higher magnification of Fig. 19. Note collapse of reticular zone and of deeper part of fasciculate zone, cells of which are crowded and appear devoid of cytoplasm. Mag. × 450.

FIG. 22.—Similar but less advanced changes in mouse Mc 803 ♂ 8 weeks old at implantation, bearing a large transplanted luteoma (Passage IIe). Mag. × 450.

FIG. 23.—Adrenal cortex of normal 6 months old male mouse. Magnification same as in Figs. 21 and 22. Mag. × 450.

FIG. 24.—Same normal adrenal gland at lower magnification (× 180) comparable to that of Fig. 20.



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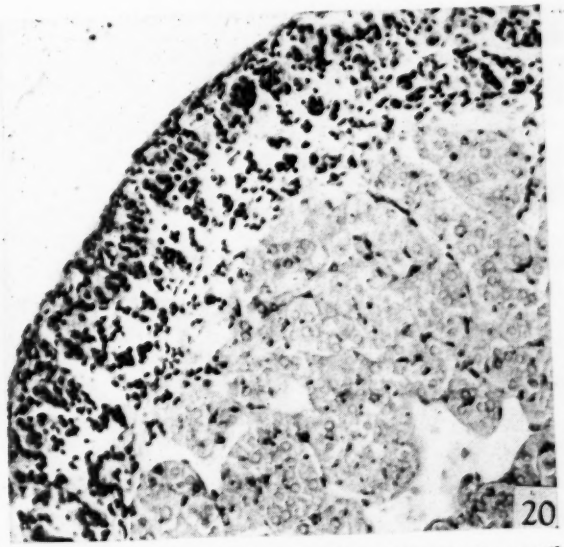

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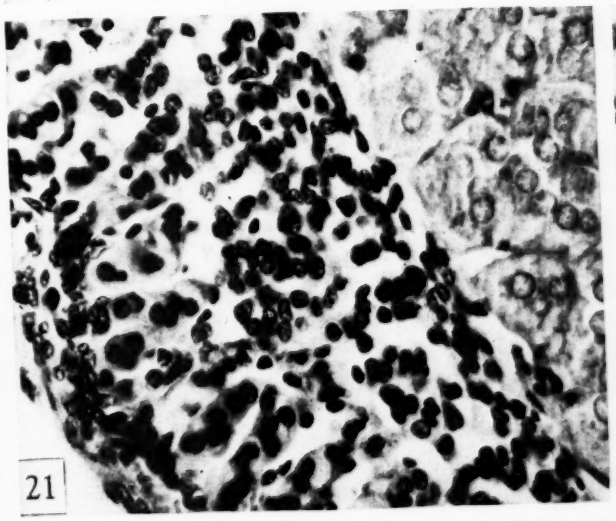

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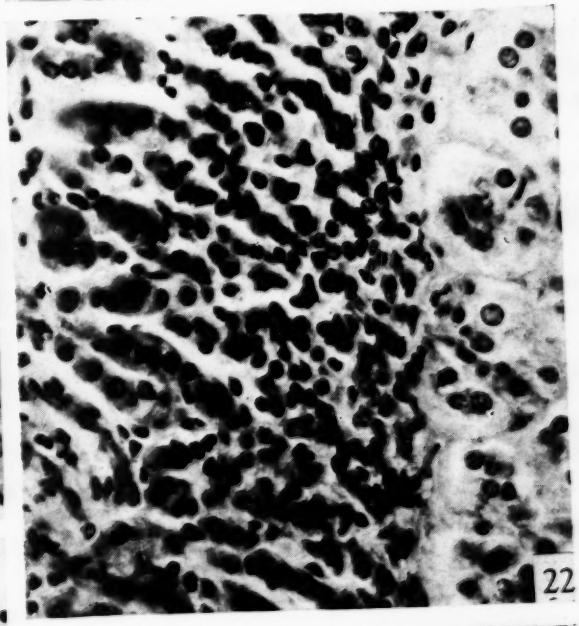

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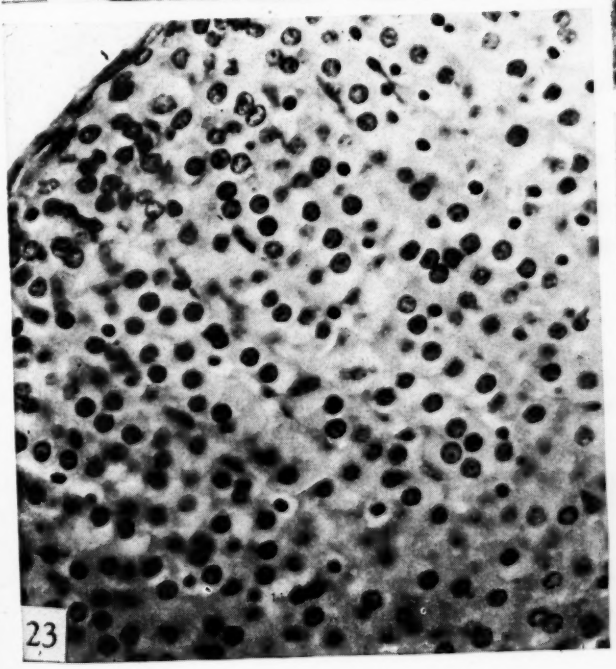

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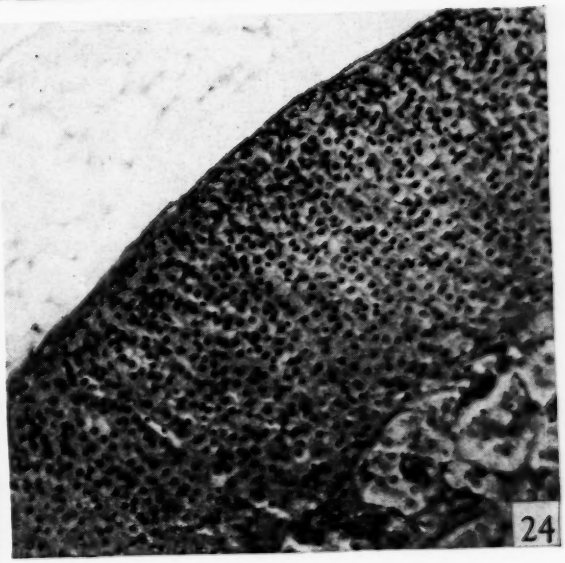

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able adrenal neoplasms composed of pure cell types might aid in the solution of this problem.

The virilizing tumors of the ovary of women have been described under such names as hypernephroma, hypernephroid tumors of the ovary, interstitioma, masculinovoblastoma and folliculoma lipidique as under adrenal tumor of the ovary and luteinoma (6).

A unique case described by Bingel (9) was associated with polycythemia and masculinization, both disappearing after removal of the tumor. Experimental granulosa cell tumors are often associated with hypervolemia which is sometimes polycythemic (21). It is possible that this case was a mixed tumor as are many of the induced ovarian tumors (18) and probably also some human luteomas accompanied by excessive bleeding, enlargement of breasts and other manifestations of feminizing inquences. A clear picture of the range of changes by secretions of one cell type can be arrived at only by obtaining a growth of one cell type in gonadectomized animals.

Identity of mouse luteoma.—To suppose that our induced luteomas originate from adrenal cell rests would imply that the ovary of every mouse contains adrenal cell rests, which following irradiation, give rise to tumors in every ovary at several places. In the adrenal gland itself tumors do not arise following general irradiation with x-rays. The possibility that luteomas are granulosa cell tumors with secondary luteinization is likewise not supported by our observations. None of our transplantable granulosa cell tumors, grafted in hundreds of mice, have ever changed into luteomas; nor have neoplastic lutein cells ever changed into granulosa cells. Such transformations are conceivable but have thus far not been demonstrated.

Relation of the adrenal gland to corpus luteum.—The morphological resemblance between luteal and adrenal cortical cells, noted as early as 1906 (34) is now generally recognized. The similarities and dissimilarities in their histological and physiological characteristics have been ably reviewed by Parkes (37), Zuckerman (50) and others.

Progesterone (7) and estrone (8) and several androgenic substances have been isolated from ox adrenals (39). The ability of progesterone and desoxycorticosterone to replace each other to a certain extent is well known. Pregnant cats survived adrenalectomy longer than did non-pregnant cats (Stewart, cited by Parkes, 37). The conditions of pregnancy and of rut exert an influence in prolonging the life of adrenalectomized dogs (40). Progesterone prolongs the life of adrenalectomized animals (22, 24, 50). Luteinizing gonadotropic hormone has a similar effect (14). Desoxycorticosterone is capable of producing progestational changes in the uterus of rabbits and of cats (Burrows 10, p. 540). The determination of the pregnandiol glucuronide levels in urine cannot be used to differentiate between a luteoma and a tumor arising from adrenal rests since both progesterone and desoxycorticosterone are metabolized to this substance (13).

Secretions of luteoma.—Complete decidualization of the endometrium and a daily output of 19 mgm. of pregnandiol has been associated with an ovarian tumor composed of lutein-like cells (Stewart, cf. 6). Attempts to isolate hormonal steroids from luteoma-bearing mice are in progress.

The evidence that the luteal cell secretes estrogen is insufficient (2, 4). None of the secondary changes induced by the experimental luteomas are caused by estrogens. The weight of evidence supports the opinion that the ovary secretes androgenic material (10), and that this androgenic activity is associated with luteinization of the ovary (26, 44). It is uncertain whether other normal ovarian cells secrete androgens. Progesterone is androgenic (24, 25) but large amounts of it are required to produce such an effect. In stimulating the secondary sex organs of castrated rats I mgm. of progesterone is equivalent to 0.03 mgm. of testosterone (33). This would imply that the secreting tissue would have to synthesize enormous amounts of progesterone to produce an androgenic effect. On the other hand, progesterone in the physiological state as secreted may be more active.

The chemical identification of a tumor cell secreting androgenic material depends on further knowledge of the secretions of the different masculinizing tumors (arrhenoblastoma adrenal cortical tumor, luteoma and testicular interstitial tumor).

Influence of progesterone on the adrenal gland.—The characteristic atrophy of the adrenal cortex in luteoma-bearing mice requires an explanation. Administration of adrenal cortical extracts causes atrophy of the adrenal cortex because of inhibition of the secretion of adrenocorticotrophic hormone by the pituitary (30). During pregnancy there are degenerative changes in the adrenal cortex (29, 45) and similar changes are readily produced by androgens. Crystalline progesterone like desoxycorticosterone produces atrophy of the adrenal cortex of rats (11) but it requires huge doses of progesterone to produce this effect (29).

In the light of the data, the atrophy of the adrenal cortex in luteoma-bearing mice is best explained as an inhibition of the secretion of adrenocorticotropin by the hypophysis by secretions of the

luteoma. The validity of this supposition remains to be tested and the mechanism of this inhibition elucidated.

The effect of progestins on the uterus.—Progesterone stimulates strongly the uterus in ovariectomized mice without preliminary treatment with estrogens (29). The uteri of mice that had been given pellets of progesterone were 30 to 40 per cent larger than those of the controls. There was generalized stimulation of all layers. The lining cells were more columnar, and there was endometrial proliferation with marked "endometrial complexity." These changes were only slightly less than those caused by normal pregnancy. More recent studies (5, 28) have shown that in castrated mice the changes induced by progesterone in the uterus affect predominantly the stroma of the submucosa. The stromal cells swell, the nuclei become rounder and more vesicular and the chromatin structure becomes more distinct. The changes differ from those produced by estrogens and may occur concurrently. The degree of change is believed to be proportional to the level of the hormonal stimulus. The administration of crystalline progesterone to rats induced progestational changes in the uterus which enlarged to 1 cm. in diameter (normal about 3 mm.).

The uterine mucosa of mice with luteoma is doubtless in a stimulated state; these mice are anestrous as indicated by the character of the vaginal epithelium. But the changes observed are not identical with those that have been described to characterize the action of administered progestins in mice. There is a conspicuous absence of stimulation of submucosal cells, and a marked stimulation of surface epithelium. This discrepancy, which is as far as we know the only one contradicting the thesis that our luteoma secretes progestins, remains to be clarified.

In the monkey, as in our mice with luteoma, there is considerable hyperplasia of the endometrium with increased tortuosity of the gland with invaginations and evaginations (50).

Effect of progesterone on estrus and ovary.—Large doses of progesterone administered to mature rats do not produce mucinification or cornification of the vaginal epithelium (43), while combined administration of estrogens and progesterone readily produces mucinification as illustrated in Fig. 18 on page 476 of Allen's *Sex and Internal Secretions* (1). Administration of crystalline progesterone to rats causes inhibition of estrus (38), or cessation of the estrous cycle with atrophy of the ovaries, which become devoid of ripe corpora lutea (43).

In luteoma-bearing mice both vaginal mucosa and ovary have a characteristic appearance. The former is atrophic 2 to 3 cell layers thick with an inner layer of cuboidal cells containing basal nuclei. The ovary is smaller than normal due to the absence of corpora lutea and of lutein cells that occupy a large part of the ovary of mature normal mice. These findings are similiar to those produced experimentally by progesterone.

Effect of progestins on seminal vesicles and prostate.—In castrated male rats the prostate and, to a lesser extent, the seminal vesicles could be maintained in weight and in secretory activity by large doses of progesterone (24). The weight of the prostate of progesterone-treated rats was 5.85 times, and that of the seminal vesicles 2.64 times that of the control litter mates. In female rats there was enlargement of the clitoris. Progesterone will also maintain spermatogenesis in hypophysectomized rats but larger quantities are required to evoke this androgenic effect.

In our experiments luteoma prevented the induction of atrophy of seminal vesicles by castration. This suggests either that the amount of progestin discharged by the tumor is large or that an androgenic substance is also produced. Progesterone will repair as well as prevent changes due to castration (24), and this occurred in luteoma-bearing mice that had been castrated before implantation.

Effect of progesterone on the body weight, deposition of fat, and on thymus.—In luteoma-bearing mice atrophy of the thymus occurred in the absence of loss of weight and in the presence of atrophy of the adrenal cortex. Atrophy of the adrenal cortex has a stimulating effect on the thymus, while both androgens and estrogens cause atrophy of this organ.

The gain in body weight after administration of progesterone was less than in the control series (11) or there was a weight loss (29). Our mice with slow-growing luteomas gained more weight than did normal control mice (particularly the females) and it is doubtful that this is fully accounted for by the bulk of the tumor they carried. At the age of 10 months these mice weighed 50 to 62 grams. Instead of cachexia the bulky growth was associated with obesity, but the gain in weight may not have been due solely to deposition of fat. Androgens cause an increase in body weight in association with nitrogen and water retention (31); and progesterone causes water retention in adrenalectomized animals (46).

During and following pregnancy women usually gain weight in excess of the weight of fetus and appendages. Among the possible causes of this gain in weight are the hormonal secretions of the corpus

luteum (41) or placenta. In mice with luteomas obesity develops with atrophy of the adrenal cortex; the latter alone has the opposite effect on body weight. This phenemenon is worthy of further study.

Dimorphism of the submaxillary gland.—The sexual dimorphism of the submaxillary gland of mice was discovered by Lacassagne (32) and independently by Fekete (15). The female type is characterized by stimulation of the terminal acinar formations which become swollen, finely granular and basophilic, while the secretory tubules are at rest, their diameter being reduced and the lumina increased. The male type is characterized by the reduction in size of the acini whose cells are clear and vacuolated. This part appears compressed by the expanded secretory tubules, the cells of which are hypertrophied and filled so densely with granules of varying sizes that the nucleus is flattened against the base, the cytoplasmic outline may be indistinct and the central canals are dilated and filled with secretion.

In female luteoma-bearing mice the appearance of the submaxillary gland resembled the male type.

Effect on the kidney and other organs.—The dimorphism of the glomeruli of mice caused by differences in shape of epithelial cells of the capsule is well known (12). The majority of female luteoma-bearing mice failed to assume the male type of glomerulus. There was, however, frequently a slight or moderate congestion of the glomeruli, but no definite parenchymatous damage.

Other changes produced by progestins have not been adequately investigated in our tumor-bearing mice. It is known that progestins produce a slight hypertrophy of the pituitary gland of mature rats (43). No marked stimulation of the mammary gland was noted and it is known that progesterone alone has no stimulating effect on the mammary gland of mature rats (43). The clitoris of mice is known to enlarge following administration of progestins and it seemed larger in some luteoma-bearing mice than in their controls.

A systematic search of the possible changes in luteoma-bearing mice on the skin, several accessory sex organs such as the prostate, fallopian tube, on other endocrine glands such as the hypophysis and thyroid, and on the potentiation of the induction of changes as placentoma (3) has not been made.

While most of the significant changes observed in the luteoma-bearing animals can be reproduced by administration of progesterone it is possible that the tumor cells secrete androgenic and other substances in addition to progesterone.

SUMMARY AND CONCLUSIONS

Luteoma is a well defined type of neoplasm of the ovary which, along with granulosa cell tumors, can be easily induced with x-rays. Neoplastic lutein cells do not revert to granulosa cells and neoplastic granulosa cells do not change into lutein cells.

The induction period of luteomas is long, and their growth is slow but progressive, ultimately resulting in bulky masses. In the course of successive passages they gain markedly in proliferative vigor. Metastases from transplanted subcutaneous growths were not observed, but intrasplenic transplants gave rise to secondary luteomas in the liver.

In tumor-bearing hosts of both sexes there was atrophy of the adrenal cortex and of the thymus. The presence of luteoma in male mice prevented atrophy of the seminal vesicle following castration, and brought about repair of castration atrophy. In female mice there was uterine hyperplasia, absence of corpora lutea in ovaries, atrophy of vaginal epithelium and enlargement of the clitoris. These changes are suggestive of secretion of progestins by the tumor. Mice with transplanted luteoma, particularly the females, gain much weight and become obese.

It is concluded that some masculinizing human ovarian tumors, which are commonly regarded as derivatives of misplaced adrenal cortical cells, are luteomas.

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Tumors Induced in Mice with *p*-Diazoaminobenzene

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The first reported investigations into the action of diazoaminobenzene (DAAB) upon animal tissues were those of Otsuka (5) and Sasaki (10). Otsuka fed 30 mice, presumably of mixed genetic type, on a diet consisting of 97 per cent unpolished rice, 2 per cent olive-oil containing 2 per cent diazoaminobenzene and 1 per cent honey. Without honey the diet seems to have been unpalatable and therefore was not consumed. Greenstuff was given as well. Twenty-eight mice survived 59 days or longer; of these 5 showed no changes in the stomach, and 2 were doubtful. The remaining 22 showed various degrees of hyperkeratosis and hyperplasia of the forestomach; 11 survived 307 to 483 days and all showed papillomatous growths. The most frequent sites of hyperplasia were in the region near the margin dividing the squamous forestomach from the glandular zone, but growths occurred elsewhere in the forestomach and, in some instances, covered the entire squamous area. Carcinoma was never seen, although numerous mitoses were found in areas of rapid growth. No significant lesions were seen in any other organ of these mice.

Otsuka refers to similar experiments in rats but states that forestomach growths were not seen so regularly. Sasaki, in a general review of carcinogenic agents tested up to that time, states that papillomatous growths were obtained in rats given DAAB orally, and includes an illustration showing a longitudinal section through a stomach which is filled by papillomatous outgrowths from the squamous epithelium but in which there is no evidence of malignancy.

Hartwell (2), in his review of substances tested for carcinogenic action up to 1939, states that Shear and Stewart had made repeated injections of crystals of DAAB subcutaneously into 36 male mice of the A strain; the majority of these mice died in less than 2 months but 17 months is given as the "duration of experiment." No tumors developed in any of these animals.

As part of a program of work on the problem of stomach tumors, it was decided to examine in this Department the type of tumors which could be induced by DAAB for comparison with those induced here by heated fats in rats (1), and by carcinogenic hydrocarbons in mice (7). Forestomach ulceropapillomas were obtained in rats maintained on a basal diet of rat cake (11), or on a vitamin A deficient diet, when 150 mgm. DAAB per 100 gm. diet were added, but these lesions were essentially the same as those obtained by Berk and Peacock (1) in rats fed overheated fats in an attempt to repeat experiments reported by Roffo (8, 9). Evidence was obtained that DAAB orally administered interfered in some way with vitamin A storage, and it is hoped to publish the details of this work later. The details of the experiments made with DAAB (paradiazoaminobenzene) in mice are described below.

EXPERIMENTAL DATA

The mice used in these experiments were all stock animals of mixed colors, either bought from a dealer or bred in this Department from mice originally obtained from the same dealer.

The *p*-diazoaminobenzene used was obtained from British Drug Houses, Ltd. It was purified by solution in ether or benzene, pouring the solution through a tower of B.D.H. alumina, concentrating the total filtrate to small bulk and precipitating by the addition of 2 volumes of petroleum ether (b. p., 60° to 80° C.). The light tan powder obtained had a melting point of 98° C.

I. Feeding Experiments

(*a*) *Rat cake diet.*—Six male and 6 female mice were given a basal diet of rat cake powder (11) to which was added 50 mgm. DAAB per 100 gms. diet. Otsuka (5) used 40 mgm. DAAB per 100 gms. diet. Two male mice and all the females were dead by 39 days, usually with degenerative changes in the liver, and acute toxic damage to kidneys affecting mainly the convoluted tubules, but with no sign

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of changes in the stomach. The renal lesions varied from cloudy swelling to hyaline degeneration of the epithelium of the proximal convoluted tubules and for the sake of brevity will be referred to as toxic nephritis. Four male mice survived 122 to 125 days, but none of them showed any stomach lesion, although 6 of 10 mice in Otsuka's series dying after the same period of feeding had shown papillomatous growths in the forestomach.

Ten additional male mice were given cake powder containing 100 mgm. DAAB per 100 gm. Of these, 7 died or were sacrificed on the 29th day; no stomach lesions were found. The other 3 mice survived 240, 241, and 294 days respectively; degenerative changes in the liver were found in all 3 mice, but the stomachs showed no abnormality.

(*b*) *Restricted diet, R.D.1.*—A special diet, R.D.1, has been used successfully in this Department for the induction of liver tumors in rats by azo-dyes. The composition of this diet is as follows:

Diet R. D. 1	Per cent
Casein	12
Potatoes (peeled, boiled)	76
Salt mixture (Glaxo, L. D. 6)	4
Dried yeast (D. C. L.)	2
Arachis oil	5
Cod liver oil	1
	100

Seven male and 3 female mice (not counting those lost by cannibalism) were given R.D.1 containing 100 mgm. DAAB per 100 gm. Four males and all 3 females were dead by 64 days; 2 males survived 92 and 95 days respectively and 1 male survived 331 days. But, while toxic nephritis and liver damage were common, none of these mice showed any significant stomach lesion.

Thus in our experience, stock mice given DAAB orally do not develop tumors of the forestomach. This is in harmony with the work of Beck and Peacock (1) who found very little reaction to orally administered, overheated fats in the stomachs of stock mice, although Wistar rats and Norwegian hooded rats frequently developed ulceropapillomas of the forestomach when given overheated fats orally, as originally claimed by Roffo (8, 9). The marked similarity of Otsuka's lesions in mouse forestomachs to those seen here in rats given DAAB or heated fats orally, suggests that his basal diet of rice, low in fat and protein, allowed of an acute shortage of some (at present unknown) factor which leads to this type of lesion. Presumably neither of our basal diets was deficient in this way as far as mice were concerned.

II. Injection Experiments

Ten male and 10 female mice, maintained on a basal diet of rat cake, were given an initial subcutaneous injection of 0.25 ml. arachis oil, containing 2 mgm. (0.8 per cent) DAAB, in the right flank; 1 male mouse died after 9 days. About 1 month after the first injection the survivors were injected with 0.5 ml. of the same strength of solution, *i.e.* 4 mgm. DAAB, subcutaneously in the left flank; another male mouse died 3 days after the second injection showing acute toxic nephritis but the first injection had only caused cystic spaces lined by fibroblasts. A third subcutaneaous injection was therefore made in 8 males and 10 females after 190 days, in the right flank, using 0.5 ml. of a 2 per cent solution, *i.e.* 10 mgm. DAAB. A sharp mortality followed, 4 males and 7 females dying within 1 week, mainly due to acute toxic nephritis. A fifth male died 30 days after the third injection, showing toxic nephritis; the kidney, liver and spleen showed marked hyaline (waxy) degeneration, but the sites of injection had only cystic spaces with fibrous walls. Another female died 48 days after the third injection; this mouse had an anaplastic mammary tumor, with metastases in the spleen, liver and kidney, but this was considered to be of spontaneous origin. Two males and 2 females survived 207 to 213 days after the third injection, *i.e.* 323 to 329 days after the first injection. Two of these mice showed degenerative changes of the liver; the kidneys were normal. One mouse had a hematoma near the site of injection but no sarcoma was seen in any mouse.

The commonest lesion in these mice injected with DAAB in arachis oil was toxic nephritis; no tumors were induced at the site of injection, nor in any organ.

III. Painting Experiments

The basal diet was again rat cake; the solvent employed was acetone (B.D.H. "analar"). Painting in the interscapular region was commenced with a 0.5 per cent solution of DAAB; after a time the strength was increased to 1 per cent, later to 2 and finally to 5 per cent.

Two males died after 15 and 17 days' painting with 0.5 per cent solution; their livers showed abscesses and focal necrosis. Two females survived 48 and 84 days respectively; only the latter showed subacute toxic nephritis, but there were degenerative changes in the liver. None of these 4 mice showed any visible sign of reaction at the site of painting. The remaining mice all received the 1 per

cent solution; one female died after 7 days (total 42 days), showing early chronic toxic nephritis and extensive liver degeneration. One male and 1 female died after 47 days at 1 per cent (total 82 days); both showed toxic nephritis and fatty degeneration of the liver, the spleens also being damaged. In these 3 mice also, no skin reaction was visible to the naked eye. One male was killed 12 days after beginning the painting with the 2 per cent solution (total 100 days); the kidneys were normal but the skin showed ulceration with some degree of thickening. Two males and 1 female died 115, 139 and 121 days respectively after start of treatment with 2 per cent solution (total 262, 286 and 209 days respectively). In all 3 mice, ulceration had occurred, with hyperplasia and hyperkeratosis; in 1 the dermis was also much thickened.

Four males, 3 females, and 1 mouse, the sex of which was unfortunately not recorded, survived to be painted with the 5 per cent solution for 101, 151, 182, 204, 147, 165, 297 and 166 days respectively (total 346, 448, 462, 484, 444, 469, 601 and 411 days respectively). Only the male that died after 448 and the female that died after 469 days (total) failed to develop horny upgrowths at the site of painting; the rest showed ulceration with considerable hyperkeratosis. In some, large numbers of deeply-staining mast cells were present just above the panniculus carnosus and hypertrichosis was also seen. The dermis was usually greatly thickened and the epidermis was hyperplastic. The female dying at 444 days (total) had large horny masses and the hyperplasia was rather disorderly. But in the female dying at 601 days and the male dying at 346 days, the lesions were considered to be malignant. In the case of the female, the first "horn," which is illustrated in Fig. 1, fell off after 556 days. Another growth formed rapidly and was removed 32 days later, the animal dying after a further 13 days. Most sections showed "pearls" of keratin, but one section showed a horny papilloma associated with a squamous carcinoma invading connective tissue; this is illustrated in Fig. 2. The growth in the male which died so very much earlier was much less striking, but it proved to be a squamous carcinoma, with spindle-cell metaplasia, invading muscle fibers. All stages from keratinizing epithelium to spindle cells were found, indicating the epithelial origin of the spindle cells (Fig. 3). The abdominal organs of these 5 mice were examined, and in 3 cases hyaline (waxy) degeneration of spleen, kidney and liver was found; toxic nephritis was present in 3 mice out of the 5 examined, and in 8 out of the 16 mice which were painted with DAAB and of which the kidneys were examined.

FIG. 1.—Large horny mass at site of painting in Mouse 564 after 556 days' painting with an acetone solution of *p*-diazoaminobenzene.

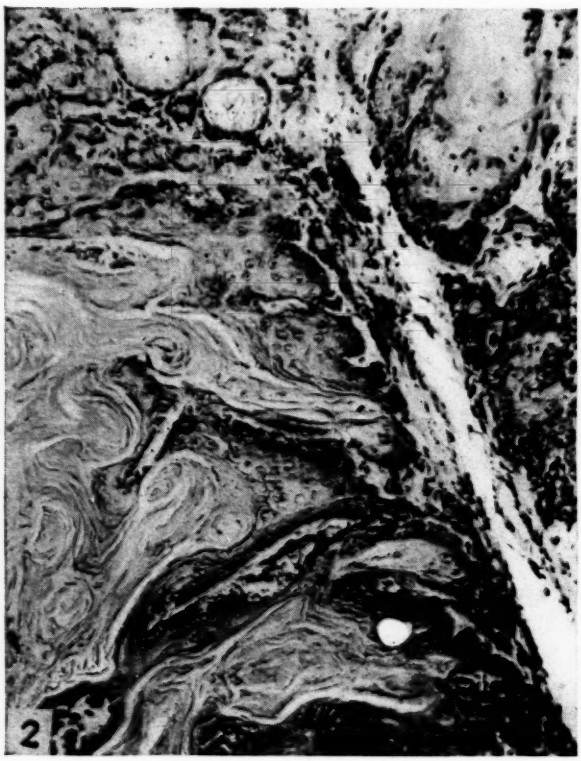
DISCUSSION

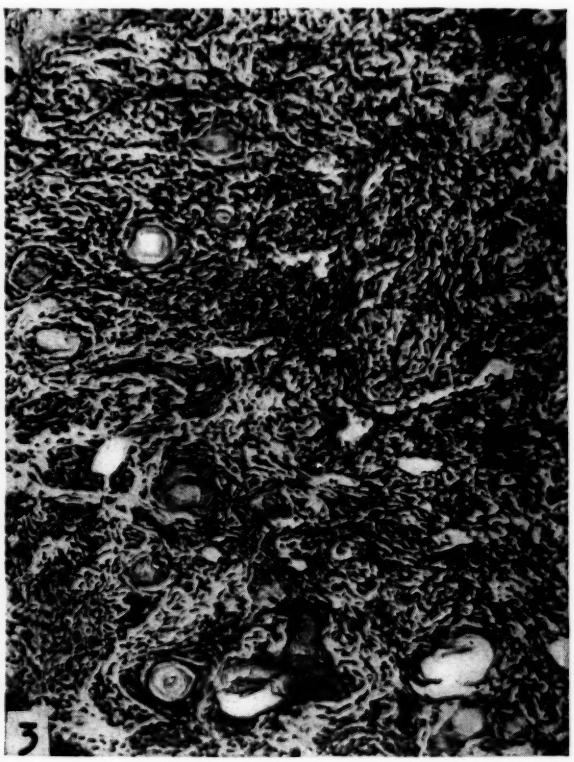
The complete absence of forestomach lesions in the mice fed with DAAB, either in rat-cake or the diet R.D.1, suggests that Otsuka's basal diet, which was very unbalanced, failed to supply some factor needed to protect stock mice against the type of forestomach lesion he saw in his mice and which we have observed in rats fed with DAAB or heated fats. We have seen similar lesions in some rats given R.D.1, and in one mouse given R.D.1 and receiving subcutaneous injections of N-N-dimethyl-*p*-aminoazobenzene (3). It would appear that the deficiency causing this type of forestomach proliferation is more easily induced in rats than in mice. Zucker, Berg and Zucker (12) have found that, whereas large quantities of vitamin A failed to protect rats against this lesion under their experimental conditions, raising the casein content of a B-complex-deficient diet from 12 to 27 per cent reduced the incidence of "rumen" lesions from 55 to 1.4 per cent. It must be pointed out that in Beck and Peacock's experiments (1) the feeding of raw carrot both prevented and cured the gastropapillomatosis otherwise noted in rats fed a diet containing overheated fats; this protective action may have resulted from improved appetite. Zucker, Berg and Zucker conclude that the lesions are due to the absence from the diet of either a labile amino-acid present in unextracted casein or to an unknown factor in crude casein. There is certainly no evidence that DAAB produces forestomach lesions *per se* in stock mice.

According to Hartwell (2), Shear and Stewart found no tumors up to 17 months after injection

of crystals of DAAB subcutaneously in strain A mice. Similarly, in the series recorded here, stock mice injected subcutaneously with DAAB in arachis oil failed to developed any tumors, at the site of injection or elsewhere, up to 329 days. DAAB, therefore, does not appear to act as a sarcogen in mice.

On the other hand, DAAB painted in acetone on the skin of stock mice leads to hyperplasia and very marked hyperkeratosis, and ultimately to squamous carcinoma which may undergo spindle-cell metaplasia as commonly occurs in mice. Thus DAAB must be regarded as a carcinogen for mouse epithelium. It seems to be clear that there is no need to demonstrate the carcinogenic action of a substance by more than one route of exhibition since a great variety of cancers is induced in rats by oral administration, and only by that route, of 2-acetylaminofluorene. Hence if a dye-intermediate, such as DAAB, is known to induce malignant tumors of the skin even in one species, it becomes an industrial hazard and must be recognized as such.





DESCRIPTION OF FIGURES 2 AND 3

FIG. 2.—Keratinizing squamous papilloma and carcinoma at the site of painting in Mouse 666, after 346 days' painting with an acetone solution of p-diazoaminobenzine. Mag. × 120. Hematoxylin and eosin stain.

FIG. 3.—Squamous carcinoma with spindle-cell metaplasia at base of horny papilloma illustrated in Fig. 1. Mag. × 120. Hematoxylin and eosin stain.

The hyaline (waxy) degeneration seen in a number of spleens from all groups of mice recorded in this paper was essentially the same as that reported by Parsons (6) after injection of nucleotides into Cba mice. We have seen the same lesion of spleens in stock mice painted or injected with 3,4,5,6-dibenzcarbazole or its *N*-methyl derivative (4) or injected with extracts of saponified bile. These degenerative changes were found also in the kidney (glomeruli) and in periportal zones of the liver. In no case have we been able to obtain metachromatic staining with methyl violet. Many mice treated with 3,4,5,6-dibenzcarbazole or its *N*-methyl derivative had numerous megakaryocytes in the spleen (4). This was also true of stock mice fed, painted or injected with DAAB, or injected with various azo dyes (3), and

presumably is due to the same stimulus created in all cases. Hyaline (waxy) degeneration around Malpighian bodies was not, however, seen in mice injected with various azo dyes (3). This latter fact, coupled with the absence of neoplastic changes in the livers of mice receiving DAAB, makes it certain that DAAB, which is readily rearranged by chemical means to yield the azo dye, *p*-aminoazobenzene, is not so altered in the body of the mouse.

SUMMARY

1. Diazoaminobenzene given orally to stock mice, in a balanced diet or in a restricted diet, failed to evoke any lesions in the foerstomach.

2. Subcutaneous injections of diazoaminobenzene in arachis oil solution caused no tumors at the site of injection in stock mice.

3. Squamous carcinoma was elicited in 2 of 8 stock mice painted interscapularly with 5 per cent diazoaminobenzene in acetone solution.

4. Toxic damage to spleen, liver and kidneys was observed when diazoaminobenzene was administered by any of the three routes employed.

5. The nature of the lesions observed in spleen, liver and kidneys makes it reasonable to suppose that diazoaminobenzene is not converted *in vivo* into *p*-aminoazobenzene.

ACKNOWLEDGEMENT

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Abstracts

Clinical and Pathological Reports

Nervous System

Correlations Between the Electroencephalogram and the Histological Structure of Gliogenous and Metastatic Brain Tumors. Greenstein, L., and Strauss, H. [New York, N. Y.] *J. Mt. Sinai Hosp.*, **12**:874-877. 1945.

Fifty-five cases of gliogenous and metastatic brain tumors were studied to determine if there was a correlation between the degree of electroencephalographic abnormality and the specific pathological nature of the tumor. The results seem to indicate that the more malignant the growth, the greater the "delta value," and that a focal per cent time delta of 40 is strongly indicative of spongioblastic or carcinomatous tumor. These findings may be of practical aid to the neurosurgeon in indicating the type of tumor to be expected in the operation.—A. C.

Spinal Extradural Arachnoid Cyst Associated with Extradural Malignancy. Cohen, I. J., *Mt. Sinai Hosp.*, *12*:116-118. 1945.

Multiple tumors of the central nervous system being rare, except in von Recklinghausen's disease, the author here presents a case in which an arachnoid cyst was present opposite the eighth thoracic vertebra and also a neuro(sympathico)blastoma at the tenth thoracic vertebra.—A. C.

The Ancestry of Neuropathology. Monsieur Antoine Louis and His "Tumeurs Fongueuses de la Dure-Mère." Courville, C. B. [Coll. of Med. Evangelists, and Los Angeles County Hosp., Los Angeles, Calif.] *Bull. Los Angeles Neurol. Soc.*. **10**:46-69. 1945.

The "fungous tumors of the dura mater" described by Antoine Louis in 1774 covered a group of lesions, largely neoplastic, that destroyed a localized portion of the cranial vault. The case histories and drawings presented by Louis suggest to the modern reader that sarcomas, meningiomas, multiple metastatic tumors, and various inflammatory and traumatic lesions were included.—M. H. P.

Eye

Orbital Tumours. Iles, A. E., and Short, A. R. [Univ. of Bristol, Bristol, England] *Brit. J. Surg.*, **31**: 147-150. 1943.

A review of 13 cases of which 4 were hemangiomas is given. Only 3 cases were malignant, and in none was there any intracranial extension. The surgical methods of approach are discussed.—E. L. K.

Breast

Histiocytosarcome du sein. Étude histo-pathologique. [Histiocyticsarcoma of the Breast. Histopathological Study.] Guibert, H. L., and Marre, Ph. [Faculty of Med., and Anticancer Center, Montpellier, France] *Bull. Assoc. franç. p. l'étude du cancer*, **30**:52-64. 1942.

A case is described of a double tumor of the breast, in which an adeno fibroma was present on the right side, and a histiocyticsarcoma, developing from the reticuloendothelial system of the gland, present on the left side.—R. J.

Duct Papillomata of the Breast. A Plea for Conservative Treatment. Wakeley, C. P. G. [King's Coll. Hosp., London England] *Brit. M. J.*, **1**:436. 1945.

Description of 3 cases.—E. L. K.

Management of Breast Tumors. McClure, R. D. and Fallis, L. S. [Henry Ford Hosp., Detroit, Mich.] *Canad. M. A. J.*, **52**:14-19. 1945.

Early detection of carcinoma of the breast can best be accomplished by periodic physical examinations in all patients over 35 years of age. When the diagnosis can be made clinically the chances of cure are not great. All breast tumors should be considered malignant until proved otherwise. The commonest are fibroadenoma, chronic cystic mastitis and carcinoma; and biopsy alone can differentiate them. When biopsy is planned the patient should be prepared mentally and the operating room in readiness for possible radical mastectomy.—M. E. H.

Angioblastoma of the Breast Complicating Pregnancy. Enticknap, J. B. [Charing Cross Hosp., London, England] *Brit. M. J.*, **2**:51. 1946.

The tumor was first noticed in the third month of the first pregnancy and was excised (weight 2.4 kgm.) two months before delivery of a healthy child. Two months later the patient died, probably from pulmonary metastases.—E. L. K.

Osteogenic Sarcoma Developing on Paget's Disease. Burr, R. C. [Kingston, Ont., Canada] *Canad. M. A. J.*, **53**:262-265. 1945.

Case report. The close association between Paget's disease and sarcoma is reviewed. In patients over 50 years of age, approximately 60% of osteogenic sarcomas of the tibia, humerus and ilium, and 100% of osteogenic sarcoma of the skull occurred on the basis of a pre-existing Paget's disease. The blood chemistry as an aid in differential diagnosis in hyperparathyroidism, Paget's disease and secondary malignancy, is discussed.—M. E. H.

Female Genital Tract

Ovarian Neoplasms. A Collective Review of the Recent Literature. Dockerty, M. B. [Mayo Clinic, Rochester, Minn.] *Surg., Gynec. & Obst., Internat. Abst.*, **81**:179-204. 1945.

The various types of ovarian neoplasms are classified

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and discussed in detail, and numerous references are sited from the literature on this subject back to 1938.—J. G. K.

Carcinoma of the Ovary Treated Preoperatively with Deep X-Ray. Report of Three Cases. PARKS, T. J. [New York, N. Y.] *Am. J. Obst. & Gynec.*, **49**:676-685. 1945.

Three cases of inoperable cystadenocarcinoma of the ovary were apparently made operable by deep x-ray therapy. In all 3 cases the neoplasm was limited to the abdominal cavity. Deep x-ray therapy caused the tumor to diminish in size, thus making the operation easier. The study indicates that it may be unwise to do a difficult primary operation since in some cases of massive infiltration it may be safer to make a biopsy, close the abdomen, treat with x-rays, and perform a second operation at a later time. Two of the patients treated by the author are alive 8 and 12 years after operation; the third died after 5 years of an apparently unrelated carcinoma.—A. K.

Adenoacanthoma of the Ovary. MELODY, G. F., FAULKNER, R. L., and STONE, S. J. [West. Reserve Univ. Sch. of Med., Cleveland, Ohio] *Am. J. Obst. & Gynec.*, **49**:691-695. 1945.

A description of two ovarian tumors containing squamous metaplasia within papillary serous cystadenocarcinoma is given.—A. K.

Arrhenoblastoma. DAUGHTRY, D. C. [Richmond Va.] *Am. J. Obst. & Gynec.*, **50**:539-541. 1945.

Case report. The patient was treated by simple surgical excision, with a resulting marked decrease of masculine features, over a period of 6 months.—A. K.

Disgerminoma of the Ovary. MITCHELL, R. [Winnipeg, Canada] *Canad. M. A. J.*, **55**:147-150. 1946.

A case of unilateral disgerminoma of the right ovary associated with pregnancy is reported. Cesarian section was first performed and a simple extirpation of the tumor was done 4½ months later. The degree of malignancy of disgerminoma is discussed.—M. E. H.

A Very Large Granulosa-Cell Tumour of the Ovary. KELSEY. H. A. [Church Missionary Society Hosp., Zaria, Nigeria] *Brit. M. J.*, **1**:922. 1945.

The tumor weighing 20 pounds was removed from a Hausa woman of Northern Nigeria who had suffered from continuous uterine bleeding for 10 months followed by complete amenorrhoea for 2 years.—E. L. K.

Enucleation of Six Ovarian Dermoid Cysts from One Woman with Conservation of the Ovaries. RUSSELL, C. S., *Brit. M. J.*, **1**:262. 1945.

Description of a case.—E. L. K.

Observation d'un cas de tumeur de Kruckenberg. [A Case of Kruckenberg's Tumor.] TISSERAND, G., GOMET, CH., and BOY, J. [Hosp. Saint-Jacques, Besançon, France] *Bull. Assoc. franç. p. l'étude du cancer*, **30**:168-171. 1942.

The case is reported because of the relative rarity of this kind of tumor. Its evolution was the famous one described by Kruckenberg.—R. J.

Pleural Effusion Associated with Ovarian Tumors (Meigs' Syndrome) SCHAFFNER, V. D., and KIRKPATRICK, T. A. [Kentville, N. S., Canada] *Canad. M. A. J.*, **55**:55-57. 1946.

Case report presented the usual findings of benign fibroma of the right ovary associated with pleural effusion in the right chest. In only 2 other instances has the pathology of the ovarian tumors been varied. In one, a thecoma of the ovary was found to be present, in the other a multilocular papillary cyst adenocarcinoma.—M. E. H.

The Early Recognition of Uterine Cancer. COSBIE, W. G. [Dept. of Obst. and Gynec., Univ. of Toronto, Toronto General Hosp., and Ontario Inst. of Radio-therapy, Toronto, Ont.] *Canad. M. A. J.*, **55**:237-240. 1946.

In the last 17 years 1,040 women have been treated for cervical cancer in the public wards of the Ontario Institute of Radio-therapy. Cancer of the cervix and uterus is diagnosed and treated earlier than it was 10 years ago and reflects the improvements that have been developed in equipment and technic. The same relative improvement is seen in the 5-year survival rate for 1940 which is 33.3% as compared with 31.7% for the whole period from 1929 to 1940. From 1938 to 1944, only 28% of patients reported to some doctor within 3 months of the onset of symptoms; in 1945, 42% did so. There has been a corresponding decrease in delay on the part of the doctor consulted in referring the patient for diagnosis and treatment.—M. E. H.

Abnormal Uterine Bleeding Past Middle Age. WILSON, T. R., and MUSSEY, R. D. [Mayo Foundation Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **19**:459-464. 1944.

Among 200 women more than 35 years of age who complained of abnormal vaginal bleeding, 80 had uterine leiomyomas or fibroids, 16 carcinoma of the cervix, 11 adenocarcinoma of the corpus uteri, 2 sarcoma of the uterus, 1 carcinoma of the ovary, 43 hyperplasia of the endometrium, and 25 atrophy of the endometrium. Among the 30 women who had bleeding after the menopause, 9 had cancer of the corpus uteri, 7 carcinoma of the cervix, 1 carcinoma of the ovary, 4 cervical polyps, 2 cervicitis, 1 senile vaginitis, and 6 no demonstrable cause for the bleeding.

The predominant symptoms of carcinoma of the cervix were metrorrhagia, occurring in 10 cases. In both cases of sarcoma of the uterus, leiomyomas were also present. The chief complaint in the case of carcinoma of the ovary was postmenopausal bleeding.—J. L. M.

Carcinoma of the Uterine Cervix. Interval Report on Treatment, Results, and Complications. SMITH, G. VAN S., and DRESSER, R. [Free Hosp. for Women, Brookline, Mass.] *Am. J. Obst. & Gynec.*, **50**:1-10. 1945.

From 1913 to 1938 progressive improvement has been noted in the results of the treatment of carcinoma of the cervix, as judged by 5 and 10 year survivals. This improvement is due to the use of radium and x-rays. Eleven hundred and eleven cases are reviewed, and deaths, early and late, following treatment and intercurrent disease are listed. Complications and their treatment are considered.—A. K.

The Wertheim Operation for Carcinoma of the Cervix. MEIGS, J. V. [Mass. General Hospital, Boston, Mass.] *Am. J. Obst. & Gynec.*, **49**:542-553. 1945.

Surgical removal of early cervical cancer is considered

to be as safe as x-ray treatment. The incidence of ureterovaginal fistulae is great, but the author feels that this can be improved.—A. K.

Myxosarcoma of Vagina Associated with Early Pregnancy. Christie F. G. S. [Sarnia, Ontario] *Am. J. Obst. & Gynec.*, **50**:533-555. 1945.

Case report.—A. K.

A Fibroma Weighing 320 Grammes Growing from the Posterior Vaginal Wall. Wallace, A. S. [Univ. Coll. Hosp., London, England] *Brit. M. J.*, **1**:631. 1945.

Description of a case.—E. L. K.

Observations on the Surgical Relief of Pain in Cancer. Turnbull, F. [Vancouver, B. C.] *Canad. M. A. J.*, **55**:241-244. 1946.

Forty-five of 136 patients with cervical cancer admitted for treatment at the British Columbia Cancer Institute during the period 1941 to 1944 had died by March 1946. The records were studied from the standpoint of pain in these patients. The group as a whole had received standard forms of modern treatment; 3 cases were eliminated because of advanced terminal cachexia; of the other 42, only 6 (14%) were spared intractable pain. The greatest incidence of intractable pain occurred 4 months before death. Among the remaining 36, 12 bore their pain for 6 months or longer; 4 suffered pain for a year. Neurosurgery (cordotomy) judiciously employed will mitigate much suffering. In the group of patients discussed, it was considered in 11, advised in 7 and performed in 4. The author feels it should have been considered in a larger percentage.—M. E. H.

Carcinoma Following Pregnancy with Spontaneous Cure. Levine, W., and Weiner, S. [Beth El Hospital, Brooklyn, N. Y.] *Am. J. Obst. & Gynec.*, **49**:778-782. 1945.

Carcinoma of undetermined origin with liver metasases and peritoneal implants is described following pregnancy in a 34 year old woman Subsequently this supposedly malignant tumor regressed. The authors suggest that secretion of large amounts of estrogen might have been responsible for the induction of the neoplasm, and that following the withdrawal of estrogen with the cessation of pregnancy the stimulus to tumor growth was gone.—A. K.

Male Genital Tract

A Large Testicular Tumor. Nightingale, H. J. [Southampton, England] *Brit. J. Surg.*, **33**:197. 1945.

The tumor, which belonged to a type of embryonal carcinoma without glycogen-containing cells, weighed 11 pounds 4 ounces. There were no metastases.—E. L. K.

Malignancy of Testicular Cancer in Man and Dogs. Innes, J. R. M. [Imperial Chem. (Pharmaceuticals) Ltd., Blackley, Manchester, England] *Brit. J. Surg.*, **31**:157-160. 1943.

Data from 20 cases in dogs are described and tabulated and the results of orchidectomy are stated. Seminomas appeared to be less malignant in dogs than in man.—E. L. K.

Importance of the Roentgen Examination in the Diagnosis of Adenoma of the Prostate. Pereira, A. [Univ. of Sao Paulo, Sao Paulo, Brazil] *Am. J. Roentgenol.*, **51**:600-613. 1944.

From his experiences with urethrocystography in 31 cases of adenoma of the prostate, the author concludes that this procedure is indispensable for determining the topographic appearance and extent of the growth. The procedure is also important in differentiating between adenomas and dysectasias of the neck of the bladder, and for revealing conditions that would make instrumental examination dangerous. Observations made by this method may suggest the treatment of choice (suprapubic or perineal operation, or endoscopic resection), may help verifying postoperative complications (structual deformities) that would retard clinical cure, and may show recurrences. The contrast agent preferred is a barium sulphate (Luxobarium) suspension. Contraindications include recent trauma resulting from instrumental examination, acute or recent gonorrhea, subacute adnexitis (prostatovesiculitis), and abscess of the prostate.— M. H. P.

Carcinoma of the Prostate in a Youth. Nicholson, N. J. [County Infirmary, Louth, Lincs, England] *Brit. J. Surg.*, **32**:533-534. 1945.

The patient, aged 15, died after supra-pubic cystostomy. Biopsy showed carcinoma of the prostate about the size of a tennis ball. There was no clinical or x-ray evidence of metastases. No autopsy was made.—E. L. K.

Some Observations on Carcinoma of the Prostate Treated with Oestrogens as Demonstrated by Serial Biopsies. Ferguson, J. D., and Pages, W. [Depts. of Surg. and Path., Central Middlesex County Hosp., England] *Brit. J. Surg.*, **33**:122-130. 1945.

The authors carried out repeated prostatic biopsies by the periurethral route in 4 cases treated with stilbestrol and one with dienoestrol during periods from 6 months to 2½ years. The carcinomas were the most common form of tumors, arising in atrophic tissue in the posterior lobe of an otherwise normal gland. The rarer form develops in a gland showing benign hypertrophic changes.

The doses given were usually 5 mgm. of stilbestrol initially and 2 mgm. subsequently, and 2 mgm. of dienoestrol reduced to 1 or 1.5 mgm. twice daily. The second specimen was taken after an interval of from 5 to 30 months. There was a consistent decrease in the number of tumor units and the majority of units also showed considerable diminution in size. Histologically the trend appeared to be a regressive change from a more cellular type of growth to a scirrhous form. There was also diminution in the size of the nuclei with concentration of chromatin and pyknosis. Another patient studied had metastases in the inguinal and axillary lymph glands. Two adjacent glands in the right axilla were selected; one of these was removed at the start of the treatment with dienoestrol and the other 3 weeks later. During this period the serum acid phosphatase had fallen from 6.2 to 0.8 units per cc. Sections stained by the method of Gomori showed considerably less acid phosphatase in the second gland.—E. L. K.

Urinary System

A Case of Benign Papilloma of the Ureter. Ottley, C. M. [New Sussex Hosp., Brighton, England] *Brit. J. Surg.*, **32**:531. 1945.

Description of a case.—E. L. K.

Carcinoma of the Bladder Associated with Presence of a Stone. INGLIS, J. McN. [Queen Elizabeth Hosp., Birmingham, England] *Brit. M. J.*, **2**:13. 1946.

The bladder contained a calculus formed around a rifle bullet. There was no history of injury to the bladder but the patient had been wounded in war in 1918. The base of the bladder was infiltrated with a ring of carcinoma "of epidermoid type."—E. L. K.

Urinary Retention in an Infant Caused by Malignant Growth. MERLIN, P. H. *Brit. M. J.*, **1**:46. 1945.

The tumor was "a highly malignant and embryonic teratoid arising from the intermediate cell mass." Its exact origin could not be stated.—E. L. K.

ORAL CAVITY AND UPPER RESPIRATORY TRACT

Salivary-gland Tumour of the Upper Lip. CURR, J. F. [Edinburgh, Scotland] *Brit. M. J.*, **2**:605. 1945.

A tumor 1 inch in diameter in the mid-line of the upper lip of a girl aged 18 had the characteristic microscopic appearance of a mixed tumor of the parotid. The literature on tumors of this gland is reviewed.—E. L. K.

Carcinoma of the Lip and Its Treatment by Radium (1928-44). CHARTERIS, A. A. [Nat. Radium Centre, Glasgow, Scotland] *Brit. M. J.*, **1**:719-721. 1946.

Observations are based on a series of 293 cases of lip cancer referred during the period 1928-44 to the Radium Department of the Western Infirmary, Glasgow. The primary lesion was on the lower lip in all but 8 cases and all but 8 of the patients were men. The Wassermann test was not made as a routine and microscopic confirmation was obtained in 75 cases. Most of the cases occurred in the fifth, sixth and seventh decades. In 51 patients the disease had spread to neighboring structures, while in 208 cases the lymph nodes were not involved. Therapy in these cases was to treat the lip and observe the node areas. Only 9% of the patients developed cervical metastases at a later date. The treatment of the primary lesion, in 242 cases, was by radium implantation in 168 cases and radium mould in 74, the former being regarded as the method of choice. With implantation, a tissue dose of 5,000 to 6,000 r, estimated 0.5 cm. from the plane of the needles, given in 168 hours was considered adequate, while with the radium mould method a dose of 6,000 r given throughout the lip over a 10 day period was sufficient. A variety of methods have been used in the treatment of cervical lymph nodes and the correct therapy is still a problem. Surgery and radium implantation are considered the most promising methods.

The results based on the observation of these 242 cases were for at least 1 year. This period is considered adequate because if failure occurs it is usually within the first year. Of 175 patients without lymph node involvement, 165 (94%) became free of disease while 53 patients with lymph node involvement or developing these subsequently, 25 (48%) were made free from disease.—M. L.

Intracranial Complications of Infections of the Nasal Air Passages and Accessory Sinuses. A Further Report on the Nature and Incidence of Lesions Observed in a Series of Thirty Thousand Autopsies. POTE, W. W. H., JR., and COURVILLE, C. B. [Los Angeles County Hosp., and Coll. of Med. Evangelists, Los Angeles, Calif.] *Bull. Los Angeles Neurol. Soc.*, **10**:114-128. 1945.

Among 160 cases of intracranial complications associated with infectious or malignant disease of the nasal cavities, studied at autopsy, there were 16 instances of malignant tumor originating from the nasal septum, nasopharynx, or nasal sinuses, or metastatic to the sinuses, with infectious intracranial complications. The growths invaded the intracranial space in 13 cases of primary cancer of the nasal air passages and accessory nasal sinuses; in the other 3 cases, cancer of the skin of the regional face came to involve the adjacent sinuses and finally led to the development of intracranial infections.—M. H. P.

Tumour of Nasal Septum (Chondrosarcoma). Operation and Recurrence. OWEN, R. D. *Proc. Roy. Soc. Med.*, **39**:362. 1946.

Description of a case.—E. L. K.

Transitional Epithelial Cell Carcinoma of the Nasopharynx. WHITELEATHER, J. E. [Baptist Memorial Hosp., Memphis, Tenn.] *Am. J. Roentgenol.*, **54**:357-369. 1945.

A series of 16 cases of carcinoma of the nasopharynx is presented with details concerning age of patient, distribution of lesions, symptoms, metastases, x-ray therapy, and survival rates. The disease affects all ages and is exceedingly malignant in children and young adults. The primary lesion is most often in, or adjacent to, the fossa of Rosenmüller; the most common sites of metastases are cervical and cranial. Radiation therapy is the accepted method of treatment. Three patients in the series have remained free of disease for periods up to 2½ years; 3 others are alive with active disease. Best results can be expected in patients treated before metastasis or intracranial extension has occurred.—E. H. Q.

Étude anatomo-pathologique avant et après roentgenthérapie. Un cas exceptionnel d'histiocytosarcome de la trachée. [Anatomical and Pathological Study before and after X-ray Therapy. An Unusual Case of Trachial Histiocyticsarcoma.] GUIBERT, H. L., BETOULIÈRES, P., and FABRE, L. [Faculty of Med., and Anticancer Center, Montpellier, France] *Bull. Assoc. franç. p. l'étude du cancer*, **31**:55-64. 1943.

This tumor was treated by x-ray therapy and a histological study made before and after the treatment. It was found that following radiotherapy most of the neoplastic elements had disappeared and were replaced by fibroid tissue.—R. J.

SALIVARY GLANDS

Mixed Salivary Gland Tumour in Palate. SIMPSON, R. R. *Proc. Roy Soc. Med.*, **39**:361. 1946.

Description of a case.—E. L. K

INTRATHORACIC TUMORS

Primary Lung Cancer in Childhood. Report of an Unusual Case. DICK, A., and MILLER, H. [Royal Northern Infirmary, Inverness, Scotland] *Brit. M. J.*, **1**:387. 1946.

In a girl aged 9 a single metastasis in the left femur attracted attention 8 months before a primary bronchial carcinoma, which ultimately involved the whole of the right lung, was detected. The histological structure of the growth in the femur was similar to that of the tumor in the lung.—E. L. K.

Carcinoma of the Lung with Nonmalignant Pleural Effusion: Recovery by Pneumonectomy. GOLDMAN, A. [St. Louis, Mo.] *J. Lab. & Clin. Med.*, **30**:361. 1945.

Abstracts of a case report.—J. G. K.

Some Unusual Thoracic Tumours. BARRETT, N. R., and BARNARD, W. G. [Surgical Units, Horton War Hosp., England] *Brit. J. Surg.*, **32**:447-457. 1945.

Reports of cases of prepericardial cyst, adenoma of the bronchus, intrathoracic lipoma, and mixed carcinoma and sarcoma of the lung is given.—E. L. K.

Mediastinal Teratoma Successfully Removed by Operation. FAWCETT, A. W., [Royal Infirmary, Sheffield, England] *Brit. M. J.*, **2**:755. 1944.

The tumor was successfully removed from a child aged 7.—E. L. K.

An Intrapericardial Teratoma in an Infant. WILLIS, R. A. [Alfred Hosp., Melbourne, Australia, and Royal Coll. of Surgeons of England, London, England] *J. Path. & Bact.*, **58**:284. 1946.

A benign, congenital, polycystic teratoma containing respiratory epithelium, gastric and intestinal mucosa, salivary and pancreatic tissue, neuroglia, striated and nonstriated muscle, cartilage and bone with marrow, was attached to the aortic trunk of an infant 10 weeks old. Four other cases of intrapericardial teratoma are recorded in the literature.—E. L. K.

GASTROINTESTINAL TRACT

Pernicious Anaemia and Carcinoma of the Oesophagus. COOKE, R. T. [Dept. of Path., Royal Infirmary, Preston, England] *Lancet*, **2**:472. 1946.

Pernicious anemia was diagnosed in this patient in 1938 and was treated successfully with liver preparations. In 1944 the patient died from carcinoma of the esophagus. Necropsy was refused. In view of the association of pernicious anemia with gastric carcinoma, this single case is published to encourage the recording of other similar conditions.—E. L. K.

Discussion on Carcinoma of Lower Oesophagus and Cardia. TANNER, N. C., ALLISON, P. R., LEWIS, I., SHORTER, A., and BARLOW, D. *Proc. Roy. Soc. Med.*, **39**:411-422. 1946.

A discussion of various forms of operation, with numerous case histories is given.—E. L. K.

Adenoacanthoma of the Stomach. STRASSMANN, G. [Metropolitan State Hosp., Waltham, Mass.] *Arch. Path.*, **41**:213-219. 1946.

Report of a case, with discussion.—J. G. K.

Argentaffin (Carcinoid) Tumours of the Small Intestine. BONAR, A. A. [Ashington Hosp., Northumberland, England] *Brit. M. J.*, **1**:391. 1946.

Description of a case of argentaffinoma of the ileum. These tumors occur most often in the appendix and ileum; in the former location they do not metastasize but in the latter metastases occur in one-fourth of the cases.—E. L. K.

Malignant Tumours of the Small Intestine. A Review of the Literature and Report of 21 Cases. FRASER, K. [Glasgow, Scotland] *Brit. J. Surg.*, **32**:479-491. 1945.

A review with 14 figures in which the pathology of these tumors is considered and a classification of the sarcomas is suggested.—E. L. K.

Confusion of Amoeboma with Carcinoma. SMYTH, M. J. [Queen Mary's Hosp., Roehampton, England] *Lancet*, **2**:376-379. 1946.

In tropical regions where amebiasis is endemic, an attack of amebic dysentery may be followed by a granulomatous condition which is often mistaken for carcinoma. Colostomy was found to lead to the disappearance of such tumor-like swellings. Where amebic dysentery is prevalent, pathological investigations should include repeated examination of feces for amebae and cysts, and biopsy examination. Moreover, anti-amebic treatment with emetine should be resorted to where radical surgery is contemplated. The occasional coexistence of ameboma and carcinoma is pointed out. Amebiasis of the skin and subcutaneous tissues at the margins of a colostomy opening is described. The ulcer is characteristic, with punched-out margins and amebae are demonstrable in histological preparations of the gangrenous skin.—L. W. P.

The Relationship of Chronic Lesions to Carcinoma of the Colon—Chronic Ulcerative Colitis. Collective Review. LYNN, D. H. [Detroit Mich.] *Surg., Gynec. & Obst., Internat. Abst.*, **81**:269-276. 1945.

Among a total of 1,467 cases of chronic ulcerative colitis, 28 patients developed carcinoma, and the average incidence was 1.9%. A single series of 95 children with chronic ulcerative colitis was studied; 6 carcinomas were found later in life, an incidence of 6.3% among this group. The authors conclude that the hypothesis that there is an increased incidence of carcinoma in chronic ulcerative colitis is an important concept.—J. G. K.

Carcinoma of the Colon Causing Acute Intestinal Obstruction in Youth of 17. SCHOLEFIELD, J. [West Middlesex County Hosp., England] *Brit. M. J.*, **2**:461. 1946.

A typical ring carcinoma of the splenic flexure was found and removed. The patient is well 7 years later. No polyps were seen in the colon and there was no family history of tumors of the large intestine.—E. L. K.

Intussusception of the Sigmoid Due to Simple Polypus with Annular Carcinoma of the Descending Colon. ROSE, B. T. [Birmingham, England] *Brit. J. Surg.*, **33**:182. 1945.

Description of a case.—E. L. K.

Carcinoma of the Rectum in Sisters. REWELL, R. E. [Guy's Hosp., Med. Sch., London, England] *Brit. M. J.*, **1**:683. 1946.

Two cases are reported. One patient aged 29 had a malignant ulcer at the pelvi-rectal junction and metastases in the liver. There were polyps throughout the colon. The other patient, aged 32, died from metastases after operation for carcinoma of the rectum. The colon showed numerous minute telangiectases.—E. L. K.

BONE AND BONE MARROW

Chondroma. ADAMS, W. S., *Proc. Roy. Soc. Med.*, **39**:363. 1946.

Description of cases.—E. L. K.